JOURNAL OF ECONOMIC ENTOMOLOGY
ENTOMOLOGICAL SOCIETY OF AMERICA

Vol. 51 December, 1958 No. 6

Reduction of Corn Yield by First-Generation Southwestern Corn Borers[1]

K. D. ARBUTHNOT, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*, and R. R. WALTON and J. S. BROOKS, *Oklahoma Agricultural Experiment Station, Stillwater*

ABSTRACT

The effect of first-generation southwestern corn borers (*Zeadiatraea grandiosella* Dyar) on the development and yield of dent and sweet corn was studied at Stillwater, Okla., in 1955. Plants in different stages of growth were manually infested with newly hatched larvae. Plant height was not significantly affected, but the weight of plants was reduced moderately by the borers. There was a greater reduction in yield of grain from small than from large corn plants. Dead heart was also more prevalent in the plants that were smallest at the time of infesting. Since small plants had greater damage than larger ones, the earliest planting consistent with good agronomic practices should avoid the greatest damage by the first generation of this borer. Dissections made at harvest did not give an accurate measurement of first-generation populations.

The damage to corn plants caused by the first generation of the southwestern corn borer (*Zeadiatraea grandiosella* Dyar) has been described by Davis *et al.* (1933), Walton & Bieberdorf (1948b), Wilbur *et al.* (1950), and Rolston (1955). Feeding on the leaves causes ragging. Feeding on the meristematic tissue of the bud causes dead heart, wherein the stalk fails to develop but secondary buds sometimes grow, producing a bushy plant. Boring into and tunneling in the stalk stunts the plant.

Walton & Bieberdorf (1948a) reported yield losses from 8.7 to 100% from infested plants, but they did not indicate the generation of the borer or the numbers of borers attacking the plants. The crop season of 1955 provided an opportunity to obtain more information on the effect of the borer on yields of dent and sweet corn. Since the natural population of the pest was very low, different levels were induced by manual infestations (Walton *et al.* 1957, Hensley & Arbuthnot 1957). The experiments were conducted near Stillwater, Okla.

The corn plants were infested with larvae hatched from eggs deposited on waxed paper by moths in laboratory cages. The eggs had been incubated in petri dishes, and seedling-corn leaves introduced to provide food for the newly hatched larvae. On the day after they hatched the larvae were taken to the field and transferred to the whorls with a camel's hair brush. The plants were treated in succession with none, two, and five borers per plant. Individual plant height to the tip of the extended leaves was recorded at the time of infesting and again later.

TESTS ON DENT CORN.—Dent corn hybrid AES 803, adapted to the locality, was planted on April 9 on upland sandy loam in hills 3 feet apart in rows 100 feet long and $3\frac{1}{2}$ feet apart. Before being infested with borers, the plants were thinned to one per hill. Growing conditions were favorable until June 23, when hail severely split the corn leaves and injured the stalks.

Each level of infestation was applied to 83 plants on May 15 and 16 and to an additional 62 plants on May 25 and 27. Forty days later, June 25 and July 5, when moth-exist holes were numerous in the stalks (Davis *et al.* 1933), alternate plants in each category were dissected, and the number of borers, dead-heart plants, and the green and dry plant weights recorded. At harvest time, August 12, the remaining plants were dissected and dead hearts, dry weight of plants, numbers of borers, and yield of grain were recorded.

Table 1 shows the effect of the borers on dent corn development. Plant growth during the 2 weeks following infestation was less as the number of borers applied increased. The dry weight after 40 days and at harvest showed differences analogous to growth rate; at both times it was greatest for plants not infested and decreased progressively to the highest borer level.

The plants on which no borers were placed gave a yield of about 20 bushels per acre. Differences in yield between levels of infestation were greater than differences in growth rate and the weight of plants. In the plants infested May 15–16, yields from the two-borer and five-borer levels were, respectively, 33 and 71% less than from the uninfested plants. In the May 25–27 infestation the yield reduction was 21% for both levels.

No dead heart developed in the plants infested on May 25–27, which averaged about 3 feet tall, but it did occur among plants infested May 15–16, which were slightly less than 2 feet tall. Within the latter group the plants that were dead-hearted averaged 5 to 8 inches shorter than those that were not affected, and more than twice as many plants were dead-hearted by five than by two borers. The dead-heart plants produced very little grain, the loss averaging 90% for the five- and 86% for the two-borer plants. However, the reductions in yield were high for the plants that did not develop dead heart—19% for the the two-borer and 62% for the five-borer plants.

Since small plants are injured most severely, cultural practices that give larger plants at the time of egg laying should reduce crop losses caused by the first generation. The earliest planting consistent with good corn culture is desirable.

[1] Accepted for publication May 2, 1958.

Table 1.—Effect of first-generation southwestern corn borers on the growth and yield of dent corn.

Number of Borers Applied per Plant	Average Plant Height (Inches)		Dry Weight per Plant (Pounds)		Yield of Grain per Plant (Pounds)	Dead Heart		
	When Infested	Increase in 2 Weeks	After 40 Days	At Harvest		Per Cent of Plants Affected	Average Height When Infested (Inches)	
							Plants Affected	Plants not Affected
Plants infested May 15–16								
0	22.6	14.8	0.35	0.54	0.21	0	0	0
2	22.3	14.4	.30	.46	.14	15	16.1	23.8
5	21.8	14.2	.24	.32	.06	36	18.0	23.4
Plants infested May 25–27								
0	35.2	17.8	0.38	0.65	0.29	0	—	35.2
2	35.6	17.0	.35	.55	.23	0	—	35.6
5	35.7	16.0	.33	.53	.23	0	—	35.7

TESTS ON SWEET CORN.—On April 21 Golden Cross Bantam sweet corn was planted in a clay loam soil. The plants were about 1 foot apart in one row in a ½-acre field, and the crop was irrigated and grew well.

Each level of infestation was applied to 27 plants on May 7–9 and to 18 additional plants on May 18. All plants were measured at the time of infesting. In the first group (May 7–9) they were measured again after 9 and 28 days and in the second group (May 18) after 19 days.

Additional data were taken on July 5 and 6, when the crop was ready for harvest as roasting ears, and the record for each plant included number of mature borers, per cent of plants that had developed dead heart, dry weight (exclusive of ears), and number and weight of ears (husk free) 5 inches or longer (table 2).

More than 30% of the plants in the test row that were not manually infested contained borers at harvest. Because of migration between plants, borer survival at each of the infestation levels could not be calculated, but the average of all levels was 51% in the May 7–9 group and 42% in the May 18 group. Dead heart was less prevalent in the plants that were larger at the time of infesting, and in both groups less when two than when five borers were applied.

Observations made when the plant heights were taken indicated that most of the migration between plants took place shortly before harvest, although the finding of dead hearts among those not infested indicated that some occurred earlier. The yield of plants not manually infested may have been affected by the borers that migrated to them. There is no evidence that the growth of small plants (infested May 7–9) was retarded by the borers within 9 days after they were applied, but after 28 days they had grown much less than those not infested, especially where five borers were applied. Growth rate in the larger plants (infested May 18) decreased as the number of borers increased, according to measurements taken 19 days after they were infested. Plant weight at harvest showed similar reductions.

The number and total weight of ears were greatly reduced in all categories of infestation. Reductions were greater when the larvae were applied to smaller plants (May 7–9), 29 and 63% by two and five borers per plant as compared with 10 and 36% when applied to larger plants (May 18). The weight per ear was also about the same in all categories of infestation.

Planting at the earliest practicable date in order that plants will be as large as possible when they are attacked will reduce the crop losses from these borers.

MEASUREMENT OF FIRST-GENERATION POPULATIONS AT HARVEST.—The results of preharvest and harvest dissections of dent corn were studied to determine whether first-generation populations could be accurately measured by making dissections only at harvest time. The pre-

Table 2.—Effect of first-generation southwestern corn borers on the growth and yield of sweet corn.

Number of Borers Applied per Plant	Number of Mature Borers per Plant	Average Plant Height (Inches)			Per Cent of Dead-Heart Plants	Dry Weight per Plant (Pounds)	Yield of Roasting Ears per Plant	
		When Infested	Increase after—				Pounds	Number
			9 or 19 days[a]	28 days				
Plants infested May 7–9								
0	1.0	13.0	13.4	39.9	4	0.54	0.59	1.52
2	1.3	13.8	14.5	32.5	23	.47	.41	1.08
5	1.8	14.1	13.6	25.4	60	.35	.21	0.56
Plants infested May 18								
0	0.4	29.8	26.1	—	0	0.43	0.50	1.18
2	1.5	28.5	23.1	—	6	.38	.38	1.06
5	1.2	27.9	21.6	—	12	.26	.24	0.65

[a] Nine days for plants infested May 7–9 and 19 days for those infested May 18.

Table 3.—First-generation infestations of the southwestern corn borer when plant dissections are made at preharvest and at harvest time.

Number of Borers Applied per Plant	Per Cent of Plants Infested		Number of Mature Borers per Plant	
	Preharvest	Harvest	Preharvest	Harvest
Plants Infested May 15–16				
0	0	22	0.0	0.2
2	82	89	1.0	1.3
5	98	97	1.0	2.3
Plants infested May 25–27				
0	0	8	0	0.1
2	84	97	1.3	1.4
5	94	100	2.4	2.7

harvest dissections were made 40 days after the borers had been applied to the plants. The results of the two dissections are given in table 3. At preharvest all borers were known to be of the first generation, represented as summer-form (spotted) sixth-instar larvae, pupae, and pupal cases. At harvest time all spotted borers and pupae and those pupal cases that appeared to be fresh were classified as of the second generation. No winter-form (immaculate) borers were found. The pupal cases that did not appear to be fresh were classified as of the first generation.

With one exception, more plants were classified as infested by the first generation at the harvest than at the preharvest dissection. In the plants to which borers were not applied none were found infested in the preharvest dissection but at harvest time 8 and 22% had borers classified as first generation. At every level more borers were classified as first generation in the harvest than in the preharvest dissection. These differences show that some of the second-generation pupae were erroneously classified as first generation.

Because of manual infestation the first-generation borers in this test were of equal age. Likewise differing from natural infestations, the second generation was smaller than that of the first generation. Since harvest-time dissections gave only approximations of the two generations in this test, they would be a less accurate basis for distinguishing the two generations in plants that were naturally infested.

References Cited

Davis, E. G., J. R. Horton, C. H. Noble, E. V. Walter, and R. A. Blanchard. 1933. The southwestern corn borer. U. S. Dept. Agric. Tech. Bull. **388.**

Hensley, S. D., and K. D. Arbuthnot. 1957. Migration of *Diatraea grandiosella* Dyar larvae on corn plants. Jour. Econ. Ent. **50:** 103.

Rolston, L. H. 1955. The southwestern corn borer in Arkansas. Arkansas Agric. Expt. Sta. Bull. **553.**

Walton, R. R., K. D. Arbuthnot, and J. S. Brooks. 1957. Further tests with insecticides to control the southwestern corn borer. Jour. Econ. Ent. **50:** 270.

Walton, R. R., and G. A. Bieberdorf. 1948a. The southwestern corn borer and its control. Oklahoma Agric. Expt. Sta. Bull. **B-321.**

Walton, R. R., and G. A. Bieberdorf. 1948b. Seasonal history of the southwestern corn borer, *Diatraea grandiosella* Dyar, in Oklahoma, and experiments on methods of control. Oklahoma Agric. Expt. Sta. Tech. Bull. **T-32.**

Wilbur, D. A., H. R. Bryson, and R. H. Painter. 1950. Southwestern corn borer in Kansas. Kansas Agric. Expt. Sta. Bull. **39.**

Insecticidal Control of *Epilachna vigintioctopunctata* (F.)[1]

G. C. SENGUPTA and N. PANDA,[2] *State Agricultural Research Station, Bhubaneswar, India*

Severe damage to brinjal (eggplant) and the potato crop is caused by *Epilachna vigintioctopunctata* (F.) (Coleoptera, Coccinellidae) which is widely distributed all over Orissa. In case of brinjal, during the months of July and August seedlings are attacked from the time of germination until they become 20 to 30 inches high. In the later stage the plants in most cases withstand the feeding of the larvae without being killed, but stunting of growth is evident.

A test to control this pest was conducted on a local variety of brinjal that was planted on July 5, 1957. The insecticide formulations tested were DDT 50% wettable powder, BHC 50% wettable powder, malathion 25% wettable powder and endrin 20% E.C. The experiment was conducted in randomized replicated plots each of which was 234 sq. ft. in area. There were five replications of each treatment. The sprays were applied with a battery knapsack sprayer on August 27, 1957, or 7 weeks after planting. Counts of the *Epilachna* grubs and pupae were made on the plants of the two central rows of each plot prior to the application of insecticides. The effects of the insecticides were determined by counting the number of *Epilachna* grubs and pupae surviving on the plants of the two central rows of each plot at the end of 48 hours after application of the insecticide. The percentage decline determined from the initial and final counts for each treated and untreated plot was transformed into angles and the critical difference at 5% level of significance in terms of angles is given at the end of table 1.

Table 1.—Percentage decline in the population of *Epilachna* grubs after spraying with different insecticides, 1957.

Insecticide and Lb. per Acre	Reduction in Infestation (Average Angle)[a]
DDT 50% W.P., 1.25	57.0
BHC 50% W.P., 1.25	49.1
Malathion 25% W.P., 2.5	65.1
Endrin 20% E.C., 1.5	67.7
Check	36.5
L.S.D. at 5% level	18.57

[a] Average of five replications in each case; 12 plants of two central rows.

CONCLUSION.—Endrin 20% E.C. sprayed with a dose of 1.5 pounds per acre was most effective. A dose of 2.5 pounds per acre of malathion 25% W.P. was also very effective. Spraying of DDT 50% wettable powder at the rate of 1.25 pounds per acre gave some control but it was inferior to endrin and malathion. BHC 50% W.P. sprayed at 1.5 pounds per acre proved to be of little value for the control of *Epilachna* grubs.

[1] Accepted for publication September 6, 1958.

[2] The authors wish to thank Dr. S. Pradhan, Insect toxicologist, Division of Entomology, Indian Agricultural Research Institute, New Delhi who kindly reviewed the manuscript.

The authors were assisted in the field work by J. Patnaik, Field Assistant.

Inhibition and Recovery of Brain Cholinesterase Activity in House Flies Poisoned with Organophosphate and Carbamate Compounds[1]

D. C. MENGLE and J. E. CASIDA,[2] *Department of Entomology, University of Wisconsin, Madison*

ABSTRACT

Seventeen organophosphate, three carbamate and one sulfamate compounds were topically applied to female house flies (*Musca domestica* (L.)) to determine the correlation between toxicity, symptomology and *in vivo* inhibition of brain cholinesterase activity. The surviving flies from an LD_{50} dose of all the compounds went through hyperactive and paralytic symptoms and definite brain cholinesterase inhibition before recovery. Except for the carbamate, Isolan, the brain cholinesterase recovered completely with 1280 minutes and with several compounds the recovery was complete 80 minutes after treatment. The time of the highest rate of mortality in the insecticide-treated population and of paralytic symptoms in the survivors was usually closely associated with the time of maximum enzyme inhibition. Malathion was the major exception where the greatest paralysis and mortality occurred after the cholinesterase activity had shown considerable recovery.

By assaying the brain cholinesterase at varying substrate concentrations it was found that at the stage of hyperactivity and early paralysis in poisoning a shift occurred in the optimum substrate concentration. This might be due to a transitory competitive stage in the cholinesterase inhibition with certain organophosphates prior to phosphorylation of the enzyme and the dephosphorylation involved in recovery. Alternative explanations are also considered.

The implication that nerve cholinesterase inhibition is the mechanism of insect poisoning by organophosphates depends upon: (a) the correlation between insect mortality and the degree of *in vivo* cholinesterase inhibition for varying doses of the insecticide; (b) the correlation between toxicity and *in vitro* anticholinesterase activity; (c) analogy with organophosphate poisoning of mammals; and (d) the failure to demonstrate other systems affected at equally low concentrations and of known physiological significance (Spencer & O'Brien 1957).

Working with parathion and 32 related compounds, Metcalf & March (1949) showed a general relationship between the *in vitro* anticholinesterase activity with bee brain homogenates and the *in vivo* toxicity to house flies and honey bees. They further demonstrated a correlation between symptoms and *in vivo* brain cholinesterase inhibition with honey bees. *In vivo* nerve cholinesterase inhibition has also shown good correlation with mortality (Chadwick & Hill 1947) and symptomology (Chamberlain & Hoskins 1951) in cockroaches poisoned with organophosphates. Malathion produced a marked cholinesterase inhibition in poisoned cockroaches and house flies but the enzyme had nearly recovered before the death of the insects (O'Brien 1956). The correlation between *in vitro* anticholinesterase activity and insect toxicity is quite good for diethyl substituted phenyl phosphates (Fukuto & Metcalf 1956). Studies with phosphoramides and phosphorothioates are less easily interpreted due to active impurities in the insecticides and the *in vivo* increase in anticholinesterase activity through oxidation reactions (Casida 1956).

Certain insecticidal carbamates are also very active *in vitro* inhibitors of insect cholinesterase (Roan & Maeda 1953, 1954, Kolbezen *et al.* 1954) and with a series of substituted phenyl N-methylcarbamates a good correlation resulted between the *in vitro* anticholinesterase activity and the insect toxicity (Kolbezen *et al.* 1954). Limited *in vivo* studies on cockroaches and house flies poisoned with N-methylcarbamates have shown less complete cholinesterase inhibition than that found with organophosphate poisoned insects, apparently due to the "reversible" nature of the carbamate inhibitors caused by *in vivo* destruction of the inhibitors and partial dissociation of the inhibitor from the enzyme during preparation of the sample for assay (Chadwick & Hill 1947, Kolbezen, *et al.* 1954, Metcalf 1955).

Many insect esterases are sensitive to organophosphates, particularly the various cholinesterases, phenylesterases, and aliesterases (Lord & Potter 1951, 1954, Hopf 1952, 1954, Casida 1955, Stringer & Fielding 1956, Metcalf *et al.* 1956, O'Brien 1957, Van Asperen 1958). The relative physiological importance of the acetylcholine esterase and the O-nitrophenyl acetate esterase in organophosphate poisoning of the American cockroach has been evaluated (Casida 1955). Studies with four organophosphates, two substrates and eight organ systems showed that the only enzyme consistently inhibited to a large extent (67 to 95%) at the prostrate stage was the acetylcholine esterase of the nerve cord (Casida 1955).

Most *in vivo* studies have utilized DFP, parathion, and para-oxon with insects. In this investigation the correlation between house fly toxicity, symptomology and the *in vivo* cholinesterase depression and recovery rates was studied with many different types of organophosphate compounds.

MATERIALS AND METHODS.—*Insects.*—Adult female house flies, *Musca domestica* (L.) (Chemical Specialties Manufacturers Association—1948 strain) were used the

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by the Research Committee of the Graduate School from funds made available by the Wisconsin Alumni Research Foundation. Accepted for publication May 5, 1958.

[2] These data were presented in part at the December 1957 meeting of the Entomological Society of America in Memphis, Tennessee. The authors are indebted to Dr. G. S. Kido and Mr. P. V. Stone of the Wisconsin Alumni Research Foundation for the majority of the insects used in these studies. The skilled technical assistance of Miss Barbara Gordon, Mrs. Judy Limpel, Mrs. Sally Krueger and Miss Mary Snell is gratefully acknowledged. The insecticides used in this study as identified by the numbers found in Figs. 2, 3, 4, and 5, were supplied by the Dow Chemical Co. (1–4), American Cyanamid Co. (5–8, 13–15), Carbide and Carbon Chemicals Co. (17), Geigy Chemical Corp. (18), Shell Chemical Corp. (9, 10), Chemagro Corp. (11), and Chipman Chemical Co. (16). The dimethyl carbamyl fluoride and dimethyl sulfamyl fluoride (compounds 19 and 20) were supplied by Dr. G. Schrader, Farbenfabriken Bayer. The *O,O*-dimethyl 2,2,2-trichloro-1-*n*-butyryloxyethyl phosphonate (compound 12) was synthesized by Arthur & Casida (1958).

first day after emergence. Prior to testing these day-old flies were fed skim milk diluted with a equal volume of water.

Compounds.—The compounds studied were greater than 95% pure based on their infra-red spectra, chromatographic characteristics and/or information provided by the supplier. These materials were dissolved in reagent grade acetone immediately prior to treatment of the insects. The names (Haller 1957) or structures of the chemicals studied are shown with the results in figures 1 to 4, inclusive.

Treatment of Insects.—The 1280 minute (21 hours and 20 minutes) LD_{50} dosage was determined for each chemical following topical application to house flies. Each fly was treated (Krueger & Casida 1957) on the ventral tip of the abdomen with 1.3 microliters of acetone containing the test compound. Flies were anesthetized with carbon dioxide during the treatment. The mortality end-point was considered to be the failure to obtain any response from the fly when probed lightly with a sharp instrument.

Ten groups of 40 flies each were removed from the population and the flies were then individually treated with the predetermined 1280 minute LD_{50}. The treatment site at the tip of the absomen was selected to minimize surface contamination of the head of the fly. In addition flies held for periods of 0 through 40 minutes prior to decapitation were stuck dorsal side down on cards treated with Tanglefoot (The Tree Tanglefoot Company, Grand Rapids, Mich.) so that the surface of the fly head could not be contaminated by contact with other insects. This precaution was not taken for flies held for periods of 80 minutes and longer during which 20 treated insects were placed in a gauze covered 1-×3-inch shell vial to which small amounts of sucrose were added to prevent starvation.

At periods of 0, 5, 10, 20, 40, 80, 160, 320, 640, and 1280 minutes after treatment, flies were decapitated with care to prevent contamination and the heads were quick frozen and held at −10° C. until assayed for cholinesterase activity. With phosphate-poisoned flies the heads were assayed within 12 hours after decapitation and this time was reduced to within 8 hours for the carbamate- and sulfamate-treated flies. Prior to decapitation the number of survivors and the symptoms of phosphate poisoning (hyperactivity or paralysis) present in 50% or more of the population were recorded.

Those groups of flies in which the phosphates or carbamates did not yield between 50 and 60% mortality after 1280 minutes were not included in the interpretation of results. The less toxic sulfamate compound was used at less than the LD_{50} level. All determinations were replicated at least three times with different populations of flies and each enzyme assay was also replicated three times, so that each point shown in the figures represents nine or more cholinesterase determinations. The carbamate compounds were quite variable in the per cent inhibition between replicates where typical means at various times after treatment with standard deviations were 81±9, 69±11, 60±7, 48±8, 43±8, 40±6, 40±7, and 40±8. The greater consistency of results with the organophosphates is indicated by means and standard deviations of 90±6, 78±6, 70±5, 55±5, 35±3, 20±4, 38±4, and 61±6.

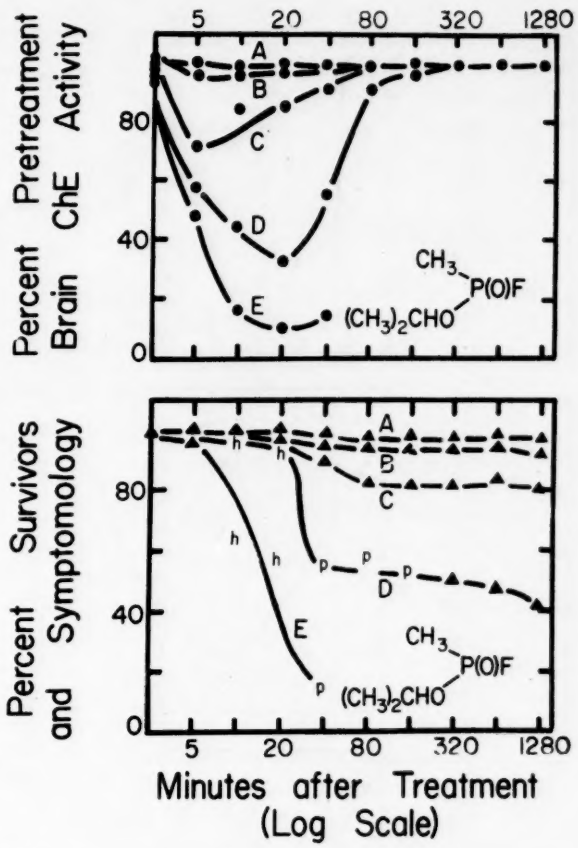


FIG. 1.—Comparison of time mortality and symptomology curves with brain cholinesterase activity of surviving house flies following topical treatment with Sarin (isopropyl methylphosphonofluoridate). Dosages are indicated by letters as follows: A. 0.1 μg./gm., B. 0.2 μg./gm., C. 0.4 μg./gm., D. 0.8μg./gm., E. 1.6 μg./gm. Symptoms observed at predetermined times after treatment were: ▲—Normal, h—Hyperactivity, p—Paralysis.

Acetylcholinesterase Determinations.—Modifications of a described manometric procedure (Casida 1955) used in this study involved the fly head homogenate being placed in the side arm to yield a final concentration of 1.50 heads per ml. after tipping, and the acetylcholine chloride in the Warburg flask to yield a final concentration of 0.01 Molar. Flasks were gassed for 10 minutes at 28° C. followed by 10 minutes equilibration at 37.5° C. prior to tipping in the enzyme. Calculations were based on the microliters of carbon dioxide evolved within 30 minutes after tipping the enzyme. Correction was made for effects other than enzymatic substrate hydrolysis and this correction was always less than 5% of the total carbon dioxide evolved.

RESULTS.—Sarin, isopropyl methylphosphonofluoridate, is one of the most toxic organophosphorus compounds to mammals (Augustinsson 1952, Saunders 1957) and house flies. The extensive studies on Sarin as an anticholinesterase agent in mammals prompted the selection of this compound to initiate studies on the correlation of *in vivo* brain cholinesterase inhibition and recovery in flies with symptomology of poisoning. The

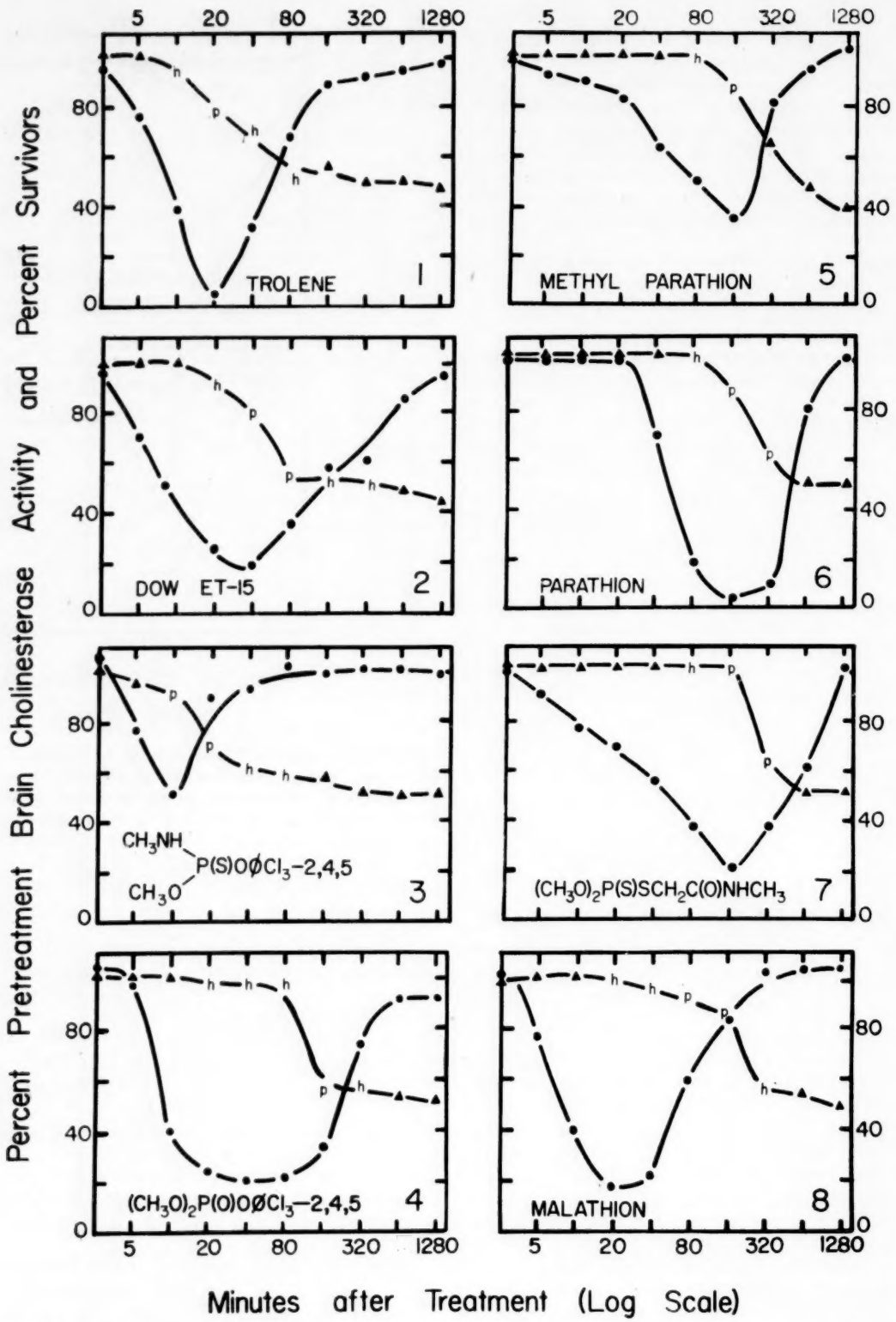


FIG. 2.—Comparison of time mortality and symptomology curves with brain cholinesterase activity of surviving house flies using several organophosphate insecticides. The 1280-minute LD_{50} dosages used were as follows: Compound 1. 1.0 μg./gm.; 2. 1.5 μg./gm.; 3. 1.5 μg./gm.; 4. 1.5 μg./gm.; 5. 0.75 μg./gm.; 6. 1.0 μg./gm.; 7. 0.38 μg./gm.; 8. 15 μg./gm.

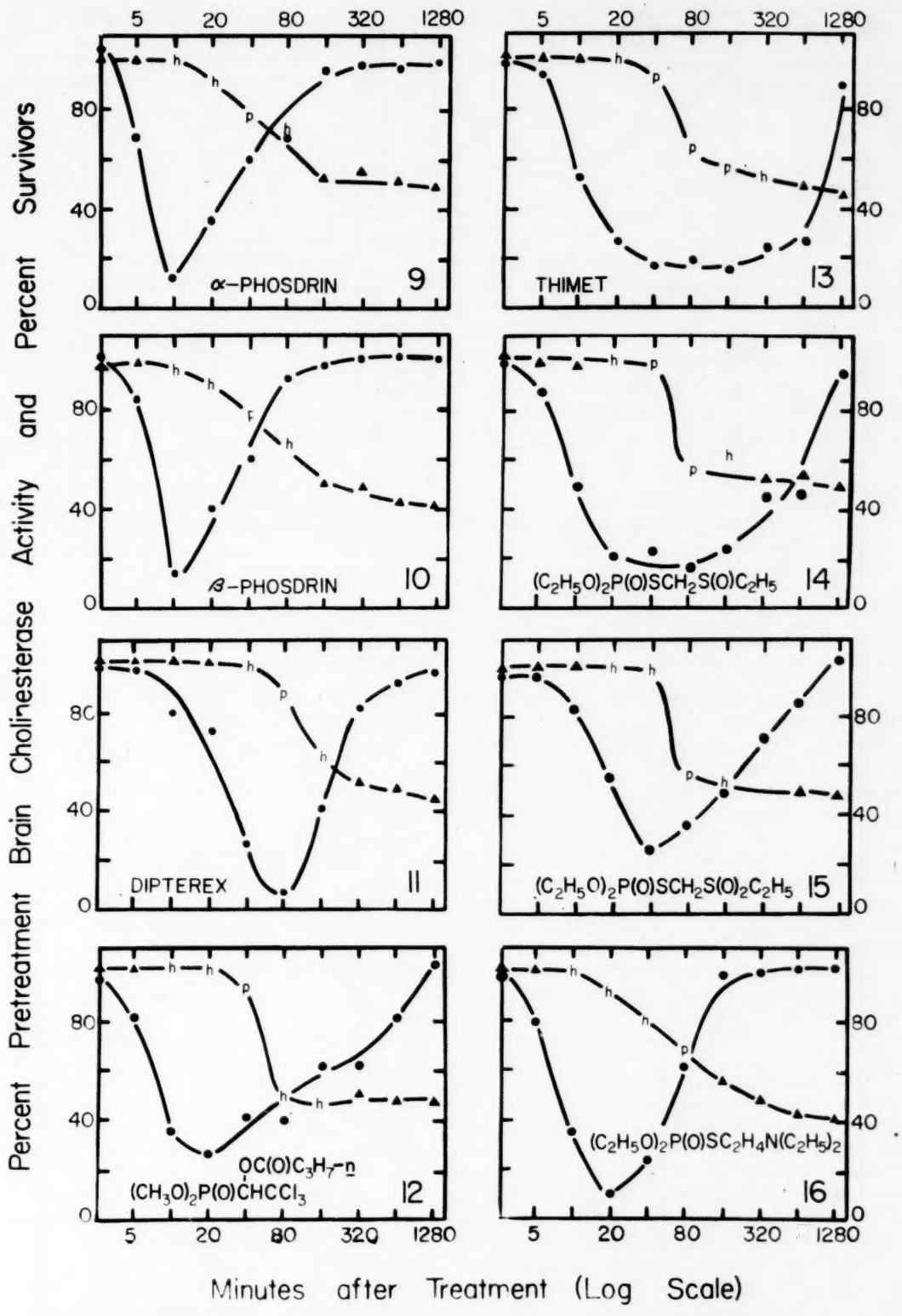


FIG. 3.—Comparison of time mortality and symptomology curves with brain cholinesterase activity of surviving house flies using several organophosphate insecticides. The 1280-minute LD_{50} dosages used were as follows: Compound 9. 0.75 μg./gm.; 10. 20 μg./gm.; 11. 6 μg./gm.; 12. 6 μg./gm.; 13. 1.5 μg./gm.; 14. 1.3 μg./gm.; 15. 2.5 μg./gm.; 16. 5.0 μg./gm.

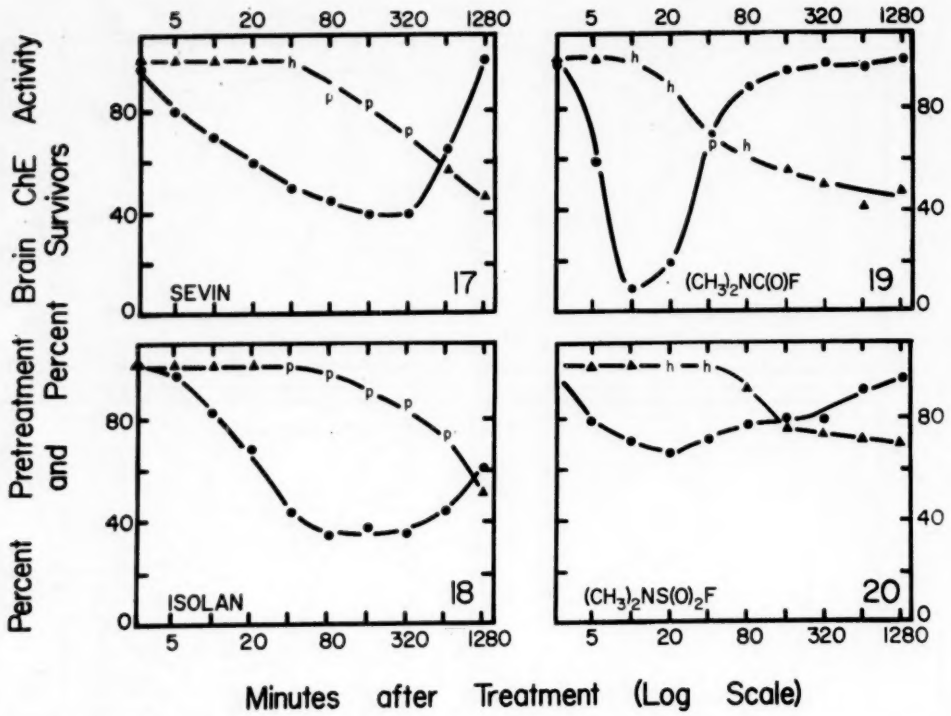


FIG. 4.—Comparison of time mortality and symptomology curves with brain cholinesterase activity of surviving house flies using carbamate and sulfamate compounds. The 1280-minute LD_{50} dosages used were as follows: Compound 17. 75 μg./gm.; 18. 15 μg./gm. 19. 45 μg./gm. Compound 20 was applied at 1000 μg./gm. which was less than the LD_{50} value.

rate and extent of *in vivo* fly brain cholinesterase depression increased with dosage of Sarin and the severity of symptoms was correspondingly greater with these higher dosages (fig. 1). Symptoms typical of organophosphate poisoning did not appear with 0.1, 0.2, and 0.4 micrograms Sarin per gram of flies and the activity did not fall below 70% of the pretreatment level. Flies treated with the predetermined LD_{50} dose showed maximum brain cholinesterase depression among the survivors with 30% of pretreatment activity in 20 minutes and thereafter recovered to normal brain cholinesterase activity by the 320 minute interval. Hyperactive symptoms were observed during and just prior to the time of minimum enzyme activity. Paralytic symptoms appeared when the enzyme level among surviving flies had recovered to 55% of normal and continued among the major portion of the survivors until the brain cholinesterase had recovered to 95% of pretreatment activity after which time the flies displayed no further symptoms of phosphate poisoning. A large proportion of the deaths occurred during the interval following the time of minimum enzyme activity thus removing from this fly population the 50% which were most susceptible to Sarin poisoning. The surviving flies of this population progressively recovered their brain cholinesterase activity in the 40- to 160-minute interval during which time no appreciable mortality occurred. It therefore appears that flies intoxicated with sublethal doses of Sarin (the 50% surviving this LD_{50} dose) undergo fairly rapid recovery of their brain cholinesterase activity. With Sarin at 1.6 micrograms per gram the mortality was such that a sufficient number of flies had been removed from the population 40 minutes after treatment to preclude further assay of the enzyme activity among the survivors.

Treatment with several organophosphate insecticides at the predetermined LD_{50} level resulted in the depression of brain cholinesterase activity among the surviving members of the population accompanied by symptoms of organophosphate poisoning (figures 2 and 3). With compounds number 1 through 7, 11, and 13 through 16, the flies started to die at about the time of maximum enzyme inhibition. With the other organophosphates (8, 9, 10, and 12) the most rapid kill occurred after the brain cholinesterase began to recover. Surviving flies from a population treated with compound 3 were observed to have their cholinesterase inhibited only to a maximum 50% of the pretreatment level. However, the time selected for the decapitation of the flies may not have coincided with the time of maximum inhibition. The failure in some cases to observe the usual hyperactive symptoms for flies passing into and recovering from the paralytic stage of organophosphate poisoning may also have resulted from a lack of coincidence of the observation times with those of maximum effect. Malathion, compound number 8, produced its maximum observed depression to 17% of normal among surviving flies 20 minutes after treatment. The enzyme activity was observed to recover to about 60% of the pretreatment level before paralytic symptoms were recorded and to about 80% before a significant number of deaths occurred. With the 17 organophosphates studied (figures 1, 2, and 3) the brain cholinesterase activity was fully recovered or nearly so in the survivors of populations 1280 minutes after application of an LD_{50} dose.

Data is presented in figure 4 on three carbamates applied at their LD_{50} dose. Dimethyl carbamyl fluoride, compound 19, gave results almost identical to those obtained with the Phosdrin isomers (numbers 9 and 10). Carbamates 17 and 18 resulted in only about 60% maximum inhibition at the times of assay and compound 18 gave the longest stage of paralytic symptoms and least cholinesterase recovery by 1280 minutes of any compound studied. With the single sulfamate studied (compound 20) hyperactive symptoms occurred with about 35% cholinesterase depression and no paralytic symptoms appeared among the majority of the population.

In the previously described experiments it was sometimes necessary to delay the enzymatic assay of the heads from organophosphate-poisoned house flies for as much as 12 hours and from carbamate and sulfamate-poisoned flies for as much as 8 hours after decapitation. To test how such a delay might affect the results, surviving flies from populations treated with either Dipterex or Thimet were decapitated at 80-, 320- and 1280-minute intervals after treatment and divided into two groups. The first group was assayed for brain cholinesterase activity immediately after decapitation while heads from the other group were held at room temperature after decapitation until the 1280 minute interval before being assayed. In addition flies found dead at the 320- and 1280-minute intervals were decapitated, divided into two groups and treated in the same manner. The results (table 1) indicate that comparatively little change in the cholinesterase inhibition is brought about by holding the heads from surviving flies for these short periods at room temperature. In the case of the brain cholinesterase activity in the heads of dead flies, only slight recovery was obtained when flies decapitated 320 minutes after treatment were held until 1280 minutes for assay. Under the normal conditions (figures 1 to 4) the heads were held at −10° C. until assayed rather than at room temperature and the change in degree of inhibition should have been even less significant.

Table 1.—The effect upon cholinesterase activity of delaying assay of heads removed from flies poisoned with Dipterex and Thimet.

Compound and Treatment to Decapitation Time in Minutes	Per Cent Pretreatment Cholinesterase Activity			
	Dead Flies Assayed		Surviving Flies Assayed	
	At Once	At 1280 Min.[a]	At Once	At 1280 Min.[a]
Dipterex				
80	—	—	21±3	36±6
320	29±8[b]	39±4	105±4	83±9
1280	29±2	—	100±5	—
Thimet				
80	—	—	10±4	15±3
320	15±4	27±5	32±6	26±6
1280	43±3	—	102±5	—

[a] Heads were held at room temperature between the time of decapitation and assay (1280 minutes).
[b] Standard deviation.

Figure 5 indicates the nature of pS curves (activity versus negative logarithm of molar substrate concentration) for brain cholinesterase from flies in various stages of poisoning with Dipterex. The enzyme from untreated flies was observed to have its maximum activity at a pS of 1.5 or at 0.013 M acetyl choline. Shortly after treatment an inhibition of activity resulted without a change in the optimum substrate concentration. At the poisoning stage of hyperactivity and early paralysis the cholinesterase activity was further depressed and the optimum substrate level was observed to shift to a higher concentration. In the same manner when flies were treated with Thimet and malathion similar shifts to higher optimum

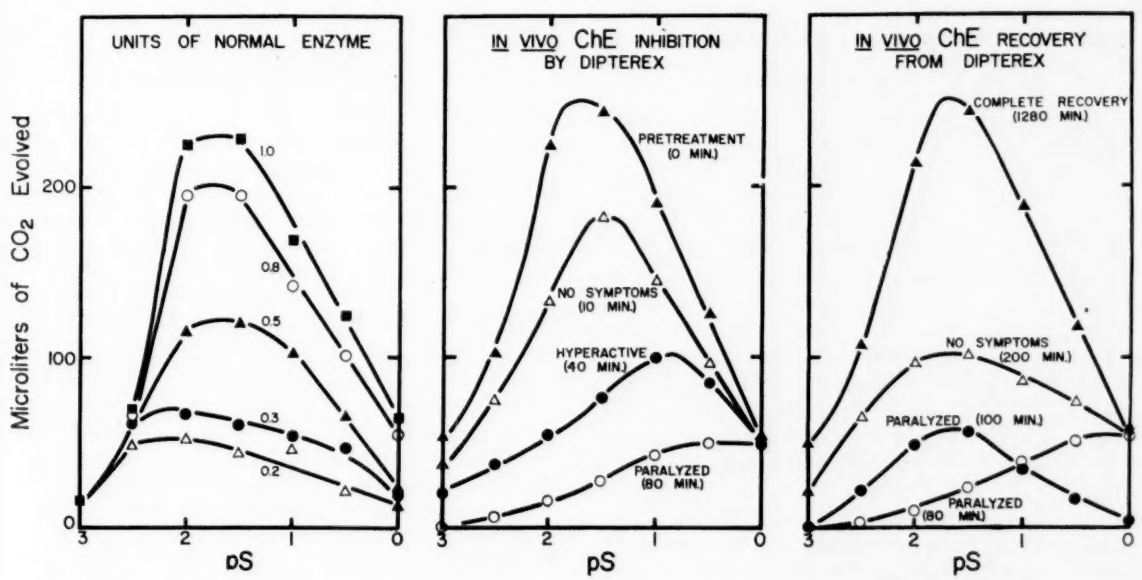


FIG. 5.—pS Curves for house fly brain cholinesterase during inhibition and recovery stages of poisoning with Dipterex compared with curves obtained by dilution of the normal enzyme concentration.

concentrations of acetylcholine were observed during stages of hyperactivity and early paralysis. With each of the three compounds tested the optimum substrate concentration began to shift back to normal while the flies were still showing paralytic symptoms. Shortly thereafter the pS optimum shifted from about 0.5 to 1.5 although the level of enzyme activity remained depressed and paralysis continued. The substrate optimum then remained constant as the symptoms of poisoning ceased and the enzyme activity returned to the pretreatment level. The length of time for the shift back to the normal substrate optimum did not correlate with either the time that enzyme activity was depressed or with the time required for the symptoms to cease. Preliminary studies using rats poisoned with Dipterex also indicate this transitory stage in which the inhibition is reversed by higher substrate concentrations.

DISCUSSION.—The insecticides studied varied greatly in the rate at which they inhibited the house fly brain cholinesterase, the maximum degree of inhibition that occurred, the time interval before recovery of the enzyme began and the rate at which this recovery took place. Definite enzyme inhibition occurred in all cases and, with the exception of the carbamate Isolan, there was complete or near complete recovery of brain cholinesterase by 1280 minutes after treatment, suggesting that the detoxification of the active inhibitor was complete within this period. It is possible, however, that small amounts of the insecticide might have been localized in sites where it was unavailable to inhibit the brain acetylcholinesterase. The phosphorothioates (compounds 1 and 13) are oxidized *in vivo* to the active antiesterase agents (compounds 4 and 15) but the rate of *in vivo* cholinesterase depression did not appear to be delayed by the necessity for this oxidation. With a majority of compounds (except 8, 9, 10 and 12) a large number of deaths in the population occurred at the time of maximum brain cholinesterase inhibition. There was a lack of any general correlation between the degree of cholinesterase inhibition and the occurrence of symptoms of organophosphate poisoning. However, paralysis occurred with all compounds and the time of paralytic symptoms with several insecticides corresponded to the time of maximum enzyme inhibition.

Acetylcholinesterase is inhibited by excess substrate and physostigmine, a reversible inhibitor of cholinesterase causes a shift in the optimal substrate concentration *in vitro* (Augustinsson 1948). This same *in vitro* shift in optimal substrate concentration has been reported with Dipterex and fly head cholinesterase (Arthur & Casida 1957). A similar shift also takes place when fly heads are assayed at various times from flies poisoned with Dipterex, this shift occurring in the early stage of paralysis (fig. 5). It appears that only a single fly head enzyme is involved in these assays since the substrate optimum shift occurs only temporarily at the time of hyperactivity and early paralysis. But two or more sites on the same enzyme might be involved with the sites being affected at different rates by the inhibitor or the inhibitor might be transferred from one site to the other before or after phosphorylation. Another explanation might be the formation of a transitory competitive stage in the inhibition. This hypothesis seems particularly attractive because of the similarity of the effect on the pS curves to that occurring with choline and physostigmine *in vitro* (Augustinsson 1948) and some recent observations with certain dimethyl phosphates in rats treated intravenously which suggest the possibility of a competitive stage in the inhibition (Vandekar & Heath 1957). This would imply a transitory competitive stage of inhibition, followed by phosphorylation of the fly head cholinesterase and enzymatic dephosphorylation for the reactivation of the cholinesterase.

The importance of excess inhibitor present in the head at the time of decapitation and reacting with the cholinesterase during homogenization and assay (Van Asperen 1958) is now being evaluated with flies at various stages of *in vivo* poisoning. Studies are also underway on several other esterases in fly heads to evaluate the contribution of their inhibition in the poisoning process.

REFERENCES CITED

Arthur, B. W., and J. E. Casida. 1957. Metabolism and selectivity of *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphanate and its acetyl and vinyl derivatives. Jour. Agric. Food Chem. **5:** 186–92.

Arthur, B. W., and J. E. Casida. 1958. Biological activity of several *O,O*-dialkyl alpha-acyloxyethyl phosphonates. Jour. Agric. Food Chem. **6:** (in press).

Augustinsson, K.-B. 1948. Cholinesterase, a study in comparative enzymology. Acta Physiol. Scand. **15,** Suppl. 52.

Augustinsson, K.-B. 1952. Organiska fosforföreningar som insektsmedel, nervgaser och enzymhämmare. Särtryck ur Svensk Kemisk Tidskrift **64:** 87–113.

Casida, J. E. 1955. Comparative enzymology of certain insect acetylesterases in relation to poisoning by organophosphorus insecticides. Biochem. Jour. **60:** 487–96.

Casida, J. E. 1956. Metabolism of organophosphorous insecticides in relation to their antiesterase activity, stability, and residual properties. Jour. Agric. Food Chem. **4:** 772–85.

Chadwick, L. E., and D. L. Hill. 1947. Inhibition of cholinesterase by diisopropyl fluorophosphate, physostigmine, and hexaethyl tetraphosphate in the roach. Jour. Neurophysiol. **10:** 235–46.

Chamberlain, W. F., and W. M. Hoskins. 1951. The inhibition of cholinesterase in the American roach by organic insecticides and related phosphorus containing compounds. Jour. Econ. Ent. **44:** 177–91.

Fukuto, Y. R., and R. L. Metcalf. 1956. Structure and insecticidal activity of some diethyl substituted phenyl phosphates. Jour. Agric. Food Chem. **4:** 930–5.

Haller, H. L. 1957. Common names of insecticides. Jour. Econ. Ent. **50:** 226–8.

Hopf, H. S. 1952. Studies on the mode of action of insecticides. I. Injection experiments on the role of cholinesterase inhibition. Ann. Appl. Biol. **39:** 193–202.

Hopf, H. S. 1954. Studies on the mode of action of insecticides. II. Inhibition of the acetylesterases of the locust nerve cord by some organic phosphoric esters. Ann. Appl. Biol. **42:** 248–60.

Kolbezen, M. T., R. L. Metcalf, and T. R. Fukuto. 1954. Insecticidal activity of carbamate cholinesterase inhibitors. Jour. Agric. Food Chem. **2:** 864–70.

Krueger, H. R., and J. E. Casida. 1957. Toxicity of fifteen organophosphorus insecticides to several insect species and to rats. Jour. Econ. Ent. **50:** 356–8.

Lord, K. A., and C. Potter. 1951. Studies on the mechanism of insecticidal action of organophosphorus compounds with particular reference to their antiesterase activity. Ann. Appl. Biol. **38:** 495–507.

Lord, K. A., and C. Potter. 1954. Organo-phosphorus insecticides, insecticidal and anti-esterase activity of organophosphorus compounds. Chem. and Indust. pp. 1214–7.

Metcalf, R. L. 1955. Organic insecticides. Interscience, N. Y.

Metcalf, R. L., and R. B. March. 1949. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. Jour. Econ. Ent. **42:** 721–8.

Metcalf, R. L., M. Maxon, T. R. Fukuto, and R. B. March. 1956. Aromatic esterases in insects. Ann. Ent. Soc. America **49:** 274–9.

O'Brien, R. D. 1956. The inhibition of cholinesterase and succinoxidase by malathion and its isomer. Jour. Econ. Ent. **49:** 484–90.

O'Brien, R. D. 1957. Esterases in the semi-intact cockroach. Ann. Ent. Soc. America **50:** 223–9.

Roan, C. C., and S. Maeda. 1953. Cholinesterase of the oriental fruit fly and its *in vitro* reactions with various insecticidal compounds. Jour. Econ. Ent. **46:** 775–9.

Roan, C. C., and S. Maeda. 1954. The cholinesterase systems of three species of fruit flies and the effects of certain insecticidal compounds on these enzymes. Jour. Econ. Ent. **47:** 507–14.

Saunders, B. C. 1957. Some aspects of the chemistry and toxic action of organic compounds containing phosphorus and fluorine. New York: Cambridge University Press.

Spencer, E. Y., and R. D. O'Brien. 1957. Chemistry and mode of action of organophosphorus insecticides. Ann. Rev. Ent. **2:** 261–78.

Stringer, A., and A. H. Fielding. 1956. The anti-esterase activity of parathion and some related organophosphorus compounds. Ann. Appl. Biol. **44:** 626–33.

Van Asperen, K. 1958. Mode of action of organophosphorus insecticides. Nature **181:** 355–6.

Vandekar, M., and D. F. Heath. 1957. The reactivation of cholinesterase after inhibition *in vivo* by some dimethyl phosphate esters. Biochem. Jour. **67:** 202–7.

The Influence of Fertilizer Applications on Populations of *Heliothis zea* (Boddie), and Certain Insect Predators[1]

PERRY L. ADKISSON,[2] *University of Missouri, Columbia*

ABSTRACT

The influence of high rates of fertilizer application upon populations of the bollworm (*Heliothis zea* (Boddie)) and certain insect predators was studied in randomized, replicated plots. One-half of each fertilizer plot received a weekly application of endrin spray from July 14 to September 4, in order to study the effect of insecticidal applications on insect populations and yields of plots receiving different fertilizer treatments.

There were no significant differences in the bollworm larval counts among the plots until mid-August, at which time highly significant differences appeared among the plots as the result of both fertilizer and spray treatments. These differences were evident for the remainder of the season.

Results indicated that the lady beetle populations were influenced by fertilizer treatments, *Orius insidious* (Say) by both fertilizer and spray treatments, *Geocoris punctipes* (Say) by spraying, and *Nabis* spp. and *Chrysopa* spp. were apparently not influenced by either the fertilizer or spray treatments.

Yields were increased by both spray and fertilizer treatments. The data indicated that as the rate of fertilizer was increased, higher returns, in terms of yield, may be expected from the use of insecticides.

Heavy applications of fertilizers usually prolong the growing season for cotton by keeping the plants succulent and fruiting later in the season. There is evidence that this practice may aggravate insect problems since (1) the longer fruiting period furnishes a source of food for a rapid buildup of large numbers of insects late in the season, and (2) highly fertilized, succulent plants may attract insects from unfertilized fields in which plants are no longer succulent or fruiting. Thus, such influences in insect populations should be known if full benefits of high fertilizer rates are to be evaluated.

Several workers have noted the influence of varying levels of soil fertility on certain insects such as the cotton aphid (*Aphis gossypii* Glov.) (McGarr 1941, 1942, Isely 1946), certain species of mirids, *e.g.* the cotton fleahopper (*Psallus seriatus* (Reut.)) (Butt *et al.* 1946, Adkisson 1957), and the bollworm (*Heliothis zea* (Boddie)) (Fletcher 1929, Thomas & Dunman 1931). Gaines (1932, 1933) reported that rate of oviposition by the bollworm moth and rate of plant growth were closely correlated and that rank, rapidly growing cotton was a preferred site. Fletcher (1941) correlated the numbers of bollworm larvae present in different fields with the moisture content of the growing tips of the cotton plants. Thus, it is evident that treatments, such as fertilizer applications, which influence plant growth may also exert considerable influence on field populations of many plant-feeding insects and their insect predators. The purpose of this investigation was to study the influence of the use of higher rates of fertilizer application on field populations of the bollworm and certain of its predators.

PROCEDURE.—A split plot design was used. The main plot treatments consisted of two rates of fertilizer application and a check. Each of the main plots was split into two sub-plots; one was regularly sprayed and one was not. Each sub-plot was 14 rows wide and 83 feet long. There were eight replications of each treatment.

The fertilizer treatments were: (1) high, 800 pounds of 12-12-12 applied at planting, plus 200 pounds of 15-15-15 and 200 pounds of ammonium nitrate applied as a side-dressing on July 9; (2) low, 300 pounds of 12-12-12 applied at planting, plus 100 pounds of 15-15-15 and 100

[1] Contribution from Missouri Agricultural Experiment Station, Journal Series No. 1891. Accepted for publication May 5, 1958.

[2] Formerly Assistant Professor, Department of Entomology, University of Missouri, now Associate Professor, Department of Entomology, Texas A. and M. College, College Station.

Table 1.—Mean numbers of bollworm larvae per 20 plants recorded in sprayed and unsprayed plots which received different rates of fertilizer.[a]

Date	Fertilizer Treatment	Unsprayed	Sprayed	Fertilizer Means
	High	0	0	0
	Low	0	0	0
July 17	Check	0	0	0
	Mean	0	0	0
	High	0.13	0.00	0.07
	Low	.00	.25	.13
July 24	Check	.00	.00	.00
	Mean	.04	.08	.06
	High	0.50	0.25	0.38
	Low	.50	.13	.32
July 31	Check	.00	.13	.07
	Mean	.33	.17	.25
	High	0.63	0.25	0.44
	Low	.38	.38	.38
August 7	Check	.25	.13	.19
	Mean	.42	.25	.34
	High	3.18	1.11	2.15
	Low	2.63	.75	1.69
August 14	Check	0.46	.34	0.40
	Mean	2.09	.73	1.41
	L.S.D. .05	0.25	.25	0.32
	L.S.D. .01	0.34	.34	0.45
	High	3.62	0.09	1.85
	Low	0.84	.17	.51
August 21	Check	1.06	.09	.58
	Mean	1.84	.12	.98
	L.S.D. .05	0.27	.27	.25
	L.S.D. .01	0.37	.37	.35
	High	4.70	1.40	3.05
	Low	4.79	.69	2.74
August 28	Check	2.12	.09	1.11
	Mean	3.87	.73	2.30
	L.S.D. .05	0.28	.28	0.73
	L.S.D. .01	0.39	.39	1.02
	High	1.96	0.21	1.08
	Low	1.72	.85	1.29
Sept. 4	Check	0.64	.00	.32
	Mean	1.44	.35	.90
	L.S.D. .05	0.26	.26	.21
	L.S.D. .01	0.36	.36	.29
	High	14.50	3.75	9.13
	Low	11.25	3.75	7.50
Total	Check	5.13	1.00	3.07
	Mean	10.29	2.83	6.57
	L.S.D. .05	1.79	1.79	2.02
	L.S.D. .01	2.43	2.43	2.80

[a] No significant differences according to F test prior to August 14.

pounds of ammonium nitrate applied as a side-dressing on July 9; and (3) the check, which received no fertilizer.

The treated plots recieved 8 weekly applications of endrin beginning July 14 and ending September 4, plus one application of 0.5 pound Guthion and 1.00 pound of DDT per acre on September 16. The first two applications of endrin were made at the rate of 0.125 pound of active ingredient per acre, the third at 0.2 pound and the last five at 0.33 pound. The sprays were prepared from emulsifiable concentrates. All applications were made by a high-clearance sprayer having one nozzle per row calibrated to deliver 2 gallons of total material per acre. The cotton was in the early bloom stage when treatment was begun. A blanket application of 0.25 pound of parathion per acre was made to all plots on September 16 for control of the cotton leaf worm, *Alabama argillacae* (Hbn.).

The plots were planted May 9, with Coker 100W registered seed. The soil was a light sandy type of low water holding capacity commonly termed "blow sand." The rainfall during the season was more than adequate and irrigation was not needed.

All records were taken from the four center rows of each plot. Insect counts were made by examining 20 plants selected at random from each plot.

Results and Discussion.—Larval counts made of the numbers of bollworms are given in table 1. There were no significant differences in the populations in the different plots until August 14, at which time there were highly significant differences in both the fertilizer and spray plots. These differences were evident for the remainder of the season. The population means for the entire season indicate that there were two or three times more larvae in the fertilized plots than in the unfertilized checks. This was true for both the unsprayed and sprayed plots; however, the sprayed fertilizer plots had a slightly smaller total infestation than the unsprayed check. The larvae in the sprayed plots were mostly in the early instars; whereas, many larvae in the unsprayed plots were in the later instars and capable of inflicting greater damage to the cotton. An examination of the means (table 1) for the entire season shows that rates of fertilizer application had as much influence on bollworm numbers as the insecti-

Table 2.—Mean numbers of beneficial insects per 20 plants recorded during the entire season in sprayed and unsprayed fertilizer plots.

Insect	Fertilizer Treatment	Unsprayed	Sprayed	Fertilizer Means
	High	10.50	10.87	10.69
	Low	8.88	9.75	9.31
Lady	Check	7.13	4.13	5.63
beetles	Mean	8.84	8.25	8.54
	L.S.D. .05	—[a]	—[a]	2.17
	High	19.88	17.12	18.50
Insidious	Low	16.88	15.13	16.00
flower	Check	11.13	7.25	9.19
bugs	Mean	15.96	13.17	14.56
	L.S.D. .05	2.62	2.62	3.71
	High	1.08	1.03	1.06
	Low	.89	.62	.75
Nabids	Check	.31	.42	.37
	Mean	.76	.69	.73
	L.S.D. .05	—[a]	—[a]	—[a]
	High	1.27	0.60	0.94
	Low	1.49	.79	1.14
Big-eyed	Check	0.79	.09	.44
bugs	Mean	1.18	.49	.84
	L.S.D. .05	0.24	.24	—[a]
	High	3.46	6.95	5.21
	Low	4.30	5.06	4.68
Lace wing	Check	3.22	3.30	3.26
flies	Mean	3.66	5.10	4.38
	L.S.D. .05	—[a]	—[a]	—[a]

[a] No significant differences according to F test.

cidal applications. Since fertilizers, particularly nitrogen, have such pronounced influence on plant growth, the difference in numbers of larvae recorded among the treatments was evidently affected by the amounts of fertilizers available to the plants. The plants responded to the different fertilizer treatments by producing varying degrees of growth, and as a result some plots were apparently more attractive to the female moths than others. Hence, the higher rates of fertilizer, by encouraging rapid plant growth, produced a more attractive environment for oviposition than the slower growing checks. It appears from these data and from those presented by Gaines (1932, 1933) and Fletcher (1941) that any treatment which encourages luxuriant plant growth would also produce a plant more attractive as an oviposition site for the bollworm.

Counts of certain beneficial insects recorded during the season (table 2) indicate that there were significant differences in their numbers among the various plots. There were significant differences in the numbers of lady beetles, *Hippodamia convergens* G.-M. and *Coleomegilla maculata langi* Timberlake, as influenced by the fertilizer treatments. The numbers of insidious flower bugs, *Orius insidiosus* (Say), were significantly influenced by both fertilizer and spray treatments. As a result of spraying, there were significant differences in the numbers of big-eyed bugs, *Geocoris punctipes* (Say), recorded among the plots. The populations of nabids, *Nabis* spp., and lace wing flies, *Chrysopa* spp., apparently were not influenced by either the fertilizer or sprays.

It should be pointed out that this experiment was surrounded on all sides by crops which, during the season, were never treated with insecticides. These crops possibly served as reservoirs from which beneficial insects could migrate into the treated plots and could, thus, affect the results. Many of the problems involved in studies of beneficial insects, such as migration between treatments, insecticide drift, and interpretation of mortality, have been pointed out by van den Bosch *et al.* (1956). These should be taken into consideration when studying the data pertaining to beneficial insects as presented in this paper.

These results, however, indicate several interesting relationships and emphasize the need for more research concerning the influence of plant nutrition and insecticidal applications on populations of beneficial insects.

Yields of seed cotton per acre are presented in table 3. Maturity, as measured by the percentage of the crop harvested at first picking, was considerably delayed by the fertilizer applications. The appearance of the plants in the sprayed plots before harvest indicated that they were maturing more rapidly than those in the unsprayed plots. However, the yield data did not support this observation.

At both pickings and in total yield there were highly significant increases due to both spray and fertilizer treatments. Spraying the fertilized plots resulted in greater increase in yield than did spraying the unfertilized checks, even though spraying did increase the yield of the check. The yield data also indicate that as the rate of fertilizer applied was increased, higher returns, in terms of yield, may be expected from insecticide applications. The increases in yield resulting from insecticide applications were nearly as great as the increases resulting from the fertilizer applications. This emphasizes the importance of insect control if full benefits from fertilizer applications are to be obtained.

Table 3.—Pounds of seed cotton per acre from sprayed and unsprayed plots receiving different rates of fertilizer.

Date	Fertilizer	Treatment		Fertilizer Means
		Unsprayed	Sprayed	
October 11	High	377.2	485.4	431.3
	Low	457.1	551.4	504.2
	Check	398.7	433.3	416.0
	Total	1233.0	1470.1	1351.5
	Spray means	413.2	490.0	450.5
	L.S.D. .05	56.1	56.1	128.0
	L.S.D. .01	76.8	76.8	177.8
December 1	High	970.3	1276.1	1123.2
	Low	977.9	1237.4	1107.7
	Check	480.4	694.6	587.5
	Total	2428.6	3208.1	2818.4
	Spray means	809.6	1069.4	939.5
	L.S.D. .05	164.3	164.3	94.3
	L.S.D. .01	233.6	233.6	131.1
Total	High	1347.5	1761.4	1554.4
	Low	1435.0	1788.8	1611.9
	Check	879.1	1127.9	1003.5
	Total	3661.6	4678.1	4169.8
	Spray means	1220.5	1559.4	1390.0
	L.S.D. .05	183.2	183.2	170.6
	L.S.D. .01	249.2	249.2	236.6

References Cited

Adkisson, Perry L. 1957. Influence of irrigation and fertilizer on populations of three species of mirids attacking cotton. United Nations FAO Plant Protect. Bull. 6(3): 33–36.

Butt, C. H., R. R. Walton, and E. E. Ivy. 1946. The cotton fleahopper in Oklahoma. Oklahoma Agric. Expt. Sta. Bull. **T-24.**

Fletcher, R. K. 1929. The uneven distribution of *Heliothis obsoleta* on cotton in Texas. Jour. Econ. Ent. **22:** 757–60.

Fletcher, R. K. 1941. The relation of moisture content of the cotton plant to oviposition by *Heliothis armigera* and to survival of growing larvae. Jour. Econ. Ent. **34:** 856–8.

Gaines, J. C. 1932. Migration and population studies of the cotton bollworm moth (*Heliothis obsoleta* Fab.). Jour. Econ. Ent. **25:** 769–72.

Gaines, J. C. 1933. Factors influencing the activities of the cotton bollworm moth. Jour. Econ. Ent. **26:** 957–62.

Isely, Dwight. 1946. The cotton aphid. Arkansas Agric. Expt. Sta. Bull. **462.**

McGarr, R. L. 1942. Relation of fertilizers to the development of the cotton aphid. Jour. Econ. Ent. **35**(4): 482–3.

McGarr, R. L. 1943. Relation of fertilizers to the development of the cotton aphid in 1941 and 1942. Jour Econ. Ent. **36**(4): 640.

Thomas, F. L., and E. W. Dunham. 1931. Factors influencing infestation in cotton by *Heliothis obsoleta* Fab. Jour. Econ. Ent. **24**(4): 815–21.

van den Bosch, R., H. T. Reynolds and E. J. Dietrick. 1956. Toxicity of widely used insecticides to beneficial insects in California cotton and alfalfa fields. Jour. Econ. Ent. **49**(3): 359–63.

Spotted Alfalfa Aphid Reaction and Injury to Resistant and Susceptible Alfalfa Clones Reciprocally Grafted[1]

T. L. Harvey and H. L. Hackerott,[2] *Fort Hays Branch, Kansas Agricultural Experiment Station, Hays*

Abstract

Spotted alfalfa aphid survival and reproduction appeared unaltered on either scions or stocks of reciprocally grafted resistant and susceptible alfalfa clones indicating that the factors responsible for resistance were not transferred. Grafting resistant plants upon susceptible stocks is suggested as a possible technique for subjecting antibiotic or non-preferred plants to aphid-injected toxin.

Since little has been reported on grafting as a possible means of transferring plant properties responsible for resistance to insects, it appeared that grafts between alfalfa clones resistant and susceptible to spotted alfalfa aphids (*Therioaphis maculata* (Buckton)) and subsequent evaluation for aphid reaction could be of value in indicating the nature of resistance.

One of the classic examples of resistance as a means of insect control involves grafting of susceptible grape scions on phylloxera-resistant rootstocks. Another example is the utilization of resistant rootstocks to control woolly apple aphids (*Eriosoma lanigerum* (Hausm.)). LePelley (1927) and Fluke (1930) reported little if any interaction between resistant and susceptible stocks and scions with respect to woolly apple aphid infestations. However, control of both woolly apple aphids and grape phylloxera was effective since rootstocks remained resistant to subterranean forms of these pests. Harland (1917) demonstrated that immune cotton scions on susceptible stocks retained immunity to leaf blister mites, but when the stocks were immune and scions susceptible, the resistance of scions appeared increased. Although increased resistance in susceptible scions may be attributed to the influence of immune stocks, Harland indicated differences in opportunities for mite infestations were not controlled.

Alfalfa plants varying from susceptible to highly resistant in reaction to spotted alfalfa aphids were readily available since resistant alfalfa plants had been selected from a wide range of susceptible varieties (Harvey & Hackerott 1956, Howe & Smith 1957, Hackerott *et al.* 1958). Since alfalfa plants may be grafted and quantitative measurements of aphid survival and fecundity can be determined, they appeared suitable for determining the interaction of resistant and susceptible stocks and scions with respect to insect resistance.

The objective of this experiment was to determine if the plant properties responsible for spotted alfalfa aphid resistance were affected by reciprocally grafting resistant and susceptible plants. In addition, it was desired to determine if aphid-injected toxin could be introduced into resistant plants by grafting antibiotic or non-preferred scions upon susceptible stocks.

The four resistant plants used in this experiment varied from nearly immune to moderately resistant. These plants were selected from the variety, Buffalo, by caged-leaf antibiosis tests on plants which survived seedling screening tests as previously described (Harvey & Hackerott 1956). The four susceptible plants were also chosen from the variety, Buffalo, and were selected to be about equally susceptible on the basis of antibiosis tests. The four resistant and the four susceptible plants were paired and reciprocal graft combinations were made between each pair. Whip grafts were made on succulent stems by the approach graft method and after the graft union became well established the desired stems were severed. Two weeks after the grafts were established spotted alfalfa aphid survival and reproduction were measured on resistant and susceptible stocks and scions and on their ungrafted clonal counterparts. This was accomplished by caging 10 apterous adults in plastic boxes on single trifoliate leaves on each stock, scion, and ungrafted clone in triplicate, and recording the number of live adults and nymphs each day for 3 days. On the grafted plants, trifoliate leaves were caged adjacent to the graft union. Entire plants involving two clones with susceptible stocks and resistant scions and two with resistant stocks and susceptible scions were exposed to aphid infestation 9 months after grafting. Relative injury to stocks and scions was observed daily for a 3-week period following infestation.

Results.—Aphid survival and reproduction were markedly less on resistant than on susceptible clones as shown in table 1. However, differences in aphid populations could not be demonstrated on plants of a given class of resistance whether tested as ungrafted clones or as scions or stocks grafted upon clones of opposite reaction to aphids.

When entire plants were exposed to aphid infestation 9 months after the grafts were established, resistant scions showed little evidence of aphid feeding or injury. This was in contrast to the top growth of susceptible stocks which was killed, except for the stems supporting resistant scions (fig. 1). Although the stems supporting resistant scions were heavily infested with aphids, only the leaves and branches were killed. Typical vein-banding symptoms were not observed on resistant scions and stocks or on susceptible scions grafted upon resistant stocks. However, vein-banding was evident on terminal trifoliate leaves of young stems of susceptible ungrafted clones and susceptible stocks with resistant scions. Susceptible scions were killed on resistant stocks; however, the resistant stocks showed no more evidence of aphid injury than ungrafted resistant clones.

Discussion.—The data presented indicate that antibiosis or non-preference properties, as defined by Painter (1951), were not transferred through the graft from resistant to susceptible scions or stocks, at least in sufficient quantities to alter susceptibility. If antibiosis or non-preference may be attributed to deficiencies in resistant

[1] Contribution No. 129, Fort Hays Branch Experiment Station and Contribution No. 709, Department of Entomology, Kansas State College, Kansas Agricultural Experiment Station, Manhattan. Accepted for publication May 12, 1958.

[2] Assistant Entomologist and Assistant Agronomist, respectively.

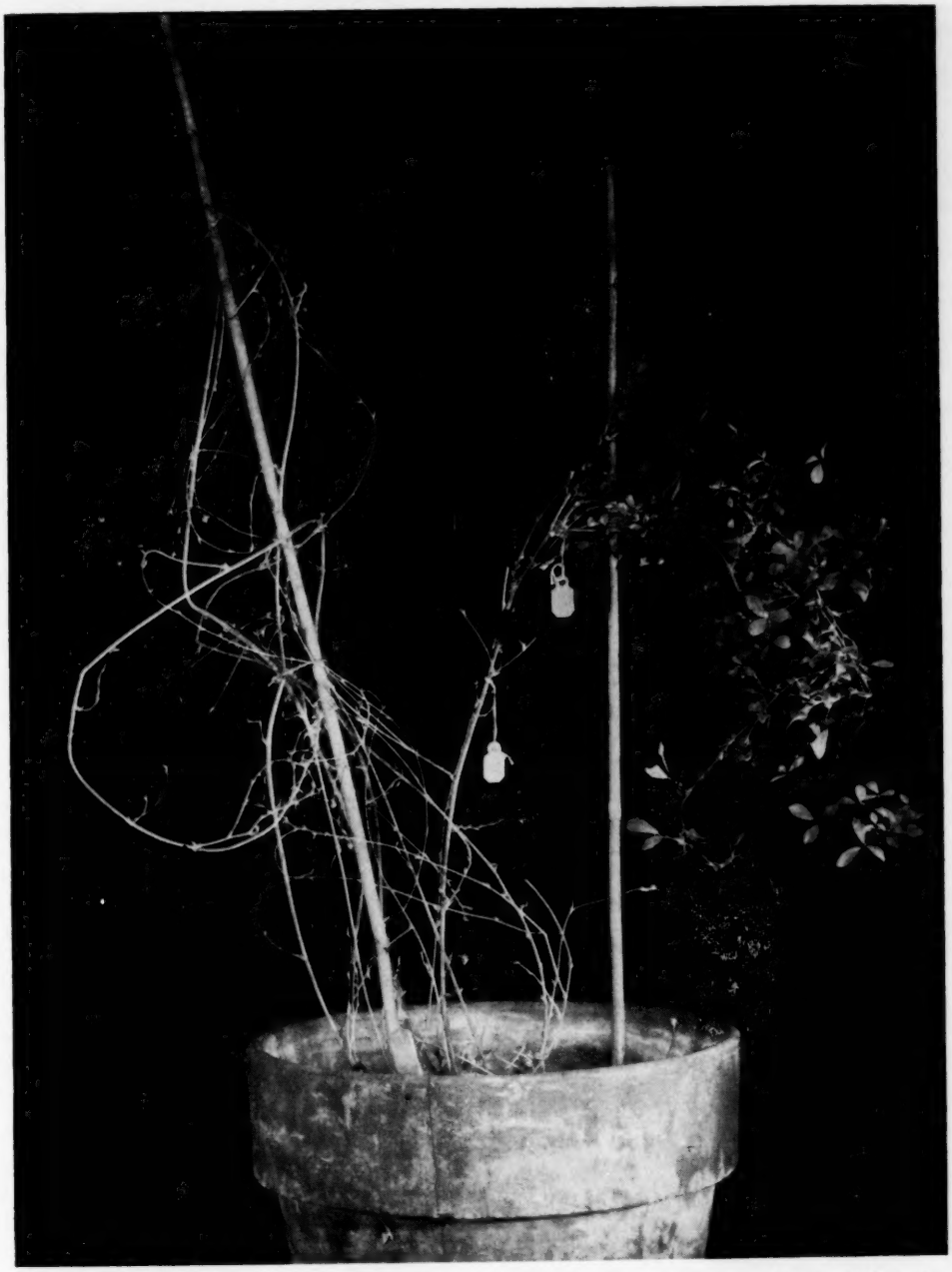


FIG. 1.—Susceptible alfalfa plant with resistant scion on right showing difference in injury 3 weeks after infestation with spotted alfalfa aphids. The graft had been established 9 months prior to infestation. The graft union is between tags.

plants, the substance or substances lacking in the resistant plants are not translocated from susceptible to resistant scions or stocks in amounts capable of altering the measurable expression of resistance. Since aphid reaction was not altered following grafting between resistant and susceptible plants, the possibility of a morphologic difference responsible for resistance between these two classes of plants was not eliminated.

An early symptom of aphid feeding on alfalfa seedlings, according to Howe & Smith (1957) and Paschke & Sylvester (1957), is yellow vein-banding of terminal leaflets. They indicated that this symptom was due to an aphid injected toxin which was systemic.

Since in this study no evidence of vein-banding was observed in resistant scions grafted upon susceptible stocks, it appears that the resistant scions either are inherently tolerant to the toxin or were not in a suitable stage of growth for expression of vein-banding symptoms. Lack of vein-banding in susceptible scions on resistant stocks may be attributed to the rapid killing of these scions by aphids. If degree of vein-banding is a valid measurement of plant tolerance to aphid-injected toxin,

Table 1.—Reaction of spotted alfalfa aphids to resistant and susceptible alfalfa clones and reciprocally grafted scions and stocks.

Classes of Clones and Types of Graft		Mean Aphid Survival and Reproduction[a] After Days Shown		
		1	2	3
Resistant as ungrafted clones	Adults	5.9	2.7	1.4
	Nymphs	4.6	2.9	2.2
stocks with susceptible scions	Adults	5.2	2.4	1.2
	Nymphs	5.6	5.1	3.5
scions upon susceptible stocks	Adults	5.3	2.7	1.8
	Nymphs	7.9	8.8	3.3
Susceptible as ungrafted clones	Adults	7.6	7.1	4.6
	Nymphs	16.7	27.8	28.2
stocks with resistant scions	Adults	7.8	7.3	6.9
	Nymphs	7.3	28.2	36.4
scions upon resistant stocks	Adults	7.8	7.6	4.9
	Nymphs	16.3	28.8	30.5

[a] Values represent mean number of live aphids per leaf on 3 leaves of 4 plants following infestation with 10 apterous adults per leaf.

these symptoms could be used to select plants tolerant to toxin. However, if the injected toxin plays a vital role in aphid feeding processes, plant tolerance to the toxin may be a mechanism responsible for antibiosis or non-preference. On the other hand, antibiosis or non-preference may be independent of tolerance to the toxin. In that case, tolerance in addition to other mechanisms of resistance may be useful, particularly in the event biotypes of aphids which are capable of feeding on antibiotic or non-preferred plants should develop.

Inasmuch as aphid populations vary with degree of antibiosis or non-preference, it is difficult to determine whether plants vary in degree of vein-banding because of differences in tolerance to toxin or because of differences in aphid feeding attributable to antibiosis or non-preference. Grafting young resistant scions upon susceptible stocks may be useful for determining tolerance to aphid-injected toxin of antibiotic or non-preferred plants via controlled aphid feeding on susceptible stocks.

References Cited

Fluke, C. L. 1930. The influence of resistant apple scions on the susceptibility of non-resistant stocks with relation to woolly aphid attacks. Jour. Econ. Ent. **23:** 741–3.

Hackerott, H. L., T. L. Harvey, E. L. Sorensen, and Reginald H. Painter. 1958. Varietal differences in survival of alfalfa seedlings infested with spotted alfalfa aphids. Agron. Jour. **50:** 139–41.

Harland, S. C. 1917. Notes on resistance to cotton leaf blister mites with special reference to budded cottons, and to cotton hybrids. West Indian Bull. **16:** 78–82.

Harvey, T. L., and H. L. Hackerott. 1956. Apparent resistance to the spotted alfalfa aphid selected from seedlings of susceptible alfalfa varieties. Jour. Econ. Ent. **49:** 289–91.

Howe, W. L., and Oliver F. Smith. 1957. Resistance to the spotted alfalfa aphid in Lahontan alfalfa. Jour. Econ. Ent. **50:** 320–4.

LePelley, Richard. 1927. Studies on the resistance of apple to the woolly aphis (*Eriosoma lanigerum* H.). Jour. Pom. and Hort. Sci. **6:** 209–41.

Painter, R. H. 1951. Insect resistance in crop plants. New York: The Macmillan Co. 520 pp.

Paschke, John D., and Edward S. Sylvester. 1957. Laboratory studies on the toxic effects of *Therioaphis maculata* (Buckton). Jour. Econ. Ent. **50:** 742–8.

The Western Tussock Moth, *Hemerocampa vetusta* (Bdv.), on Citrus in Southern California[1]

E. L. Atkins, Jr.,[2] *University of California Citrus Experiment Station, Riverside*

Abstract

Larvae of the western tussock moth, *Hemerocampa vetusta* (Bdv.), caused economic losses in 10,000 acres of oranges during the springs of 1956 and 1957 in parts of southern California. Population levels were as high as 1,000 larvae per tree. The larvae may destroy the new spring flush of growth, the newly set fruit, and scar maturing oranges so that the fruit drops or the grade is lowered. This moth has one generation a year. The wingless females lay their eggs on the trees in masses from May to July; the eggs hatch during March and April of the following year. The average length of life cycle is 75 days. A population level of 100 caterpillars per orange tree, or an average of one egg mass per tree, is considered the economic level. Six parasite species, one hyperparasite, and two predator species were collected during the years 1956 and 1957 and were observed to reduce the population approximately 50% in some groves. Chemical control studies showed that parathion, TDE (DDD), DDT, Dilan, Guthion, Sevin (*N*-methyl-1-naphthyl carbamate), methyl parathion, and Phosdrin gave excellent control; nine other pesticides did not provide adequate control. Parathion, TDE, and DDT are now registered for use on citrus and are recommended for control of the western tussock moth when applied as complete foliage sprays at the rate of 2, 5, and 5 pounds, respectively, of actual toxicant per acre.

Larvae of the western tussock moth, *Hemerocampa vetusta* (Bdv.), caused serious economic losses in two adjacent 20-acre orange groves at West Covina, Los Angeles County, in early June, 1956 (Atkins 1957a). Lighter infestations were observed on approximately 100 acres at Puente and in the San Fernando Valley. There were also reports of infestations ranging from a trace to

[1] Paper No. 1043, University of California Citrus Experiment Station, Riverside, California. Presented in part at the Forty-first Annual Meeting of the Pacific Branch of the Entomological Society of America at Portland, Oregon, June 26–28, 1957. Accepted for publication May 15, 1958.

The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

[2] The author acknowledges the valuable assistance of J. C. Ortega in applying the pesticides.

medium degree in La Habra and Brea in Orange County (Osborn 1956).

In 1957 this insect was probably the most economically important orangeworm pest on citrus. In addition to the areas of heavy infestation reported in 1956 near Covina and Puente, Los Angeles County, there were economically infested areas of 1,500 acres near Corona, Riverside County, 100 acres at Simi, Ventura County, and 150 acres at Placentia, Orange County. Population levels in these infested groves ranged from 30 to 1,000 caterpillars per tree (Atkins 1957b). Approximately 10,000 acres of citrus in southern California were infested in 1957.

ECONOMIC IMPORTANCE.—In heavy infestations the tussock moth is capable of destroying all the new spring flush of growth; it may also eat into the newly set fruit, destroying as much as 80%; it may also eat into some of the maturing Valencia and navel oranges that have not been picked and cause dropping and degrading of surface-scarred fruit. The entire sequence of damage occurs within a 6-week period. A preliminary egg-mass survey indicates an even higher amount of economic loss from the larvae of this moth during the spring of 1958 (Atkins 1957b).

HOST PLANTS.—This caterpillar is commonly found on live oak trees and on the yellow perennial lupine, *Lupinus arboreus*, in California (Volck 1907). In orchards it is a common pest on apple, cherry, prune, walnut (California black and Persian), and avocado trees (Forbes 1957), as well as on orange trees. It is also a pest on ornamentals (Osborn 1956).

LIFE CYCLE.—There is only one generation of this tussock moth per year, in contrast to as many as 10 generations with some of the other orangeworms. Hatching commences in orange groves about the time the spring flush of foliage is coming out and expanding; however, there is often great variability in the time of hatching. In 1957 in one grove, the eggs began hatching on March 8, and hatching was completed on April 2, a period of 25 days. The caterpillars require 45 to 60 days for development, the length of the period depending on the temperature. The pupal stage lasts from 13 to 24 days from the last larval molt and ends with the emergence of the adult moths. The average length of time from egg hatching to adult emergence is 74 to 75 days. The moths are emerging and are present in the groves in May, June, and July. Fertilization usually occurs immediately after the wingless female emerges, and oviposition is completed within 3 to 4 days. Each female moth lays 125 to 300 eggs in a single egg mass, which does not hatch until the following spring.

The habits of the western tussock moth have been reported in earlier papers by Volck (1907) and Atkins (1957a) and are not repeated here.

ECONOMIC LEVEL.—Careful observations of infestations of the western tussock moth in citrus groves during 1956 and 1957 indicate that a population of approximately 100 caterpillars per tree will always cause economic losses if the larvae are allowed to become fully grown. In other words, a population of 100 caterpillars per tree is the level at which chemical control will always be practical. Since it is almost impossible to determine the number of larvae present until they are causing serious damage at the economic population level or until they are too large to be easily controlled with insecticides, the following method is suggested for determining the expected population level before egg hatching begins. This 6-month period is from August through January. Economic losses may be impending when an average of one unparasitized or unpredatized egg mass is found per tree. Eight to 10 trees should be inspected per acre or a minimum of 25 trees for each 10-acre block of citrus.

The fully grown larvae select somewhat protected places in which to spin their cocoons and pupate. Consequently, approximately 75% of the cocoons are found on the trunk and main scaffold branches of the tree. Cocoons may be found anywhere on the tree, however, and also on propping stakes, on picking boxes left in the grove or stored nearby, on orchard heaters, in irrigation stands, and on fence posts. Since the egg masses are usually deposited on the pupal cases from which the wingless female moths emerge (Atkins 1957a), a careful inspection of the trunk and scaffold branches on all sides of a tree by an observer standing on the ground will reveal most of the egg masses in these locations and will give an adequate index of the potential population level expected.

To date, no tussock moth egg parasites have been observed on citrus. However, the dermestid egg-mass predator *Trogoderma sternale* Jayne is very common in the Covina area, where it has destroyed as much as 50% of the egg masses. Adult dermestids are common from March through September, and larvae have been observed in every month of the year. Predatization of the egg masses may be easily determined by lightly scraping the surface of the mass with a finger nail, pencil point, or knife blade. If the egg mass has been infested by this predator, the surface will scrape off easily and reveal the predation, whereas unpredatized egg masses will not scrape off. Of course, it is desirable to make this determination early in the year, during January and February, before egg hatching commences, but still late enough in the egg-stage period to have permitted maximum predation.

NATURAL CONTROL.—Six parasite species, one hyperparasite, and two predator species were taken during observations in 1956 and 1957. These were identified by the Insect Identification and Parasite Introduction Laboratories of the United States Department of Agriculture, Washington, D. C., or by the California State Department of Agriculture at Sacramento, California, and are listed in table 1.

This impressive list of beneficial insects accounted for the reduction of approximately 50% of the tussock moth population in 1956 and 1957 in some groves. The parasites and predators lessen but do not prevent current larval damage and do reduce the carryover of the species for the next year.

CHEMICAL CONTROL.—In 1956, preliminary control trials were initiated when most of the larvae were in the last instar (Atkins 1957a). DDT used at the rate of 10 pounds of 50% wettable powder in 800 gallons of water per acre was applied by hand spraying with a conventional spray rig. The DDT provided excellent control of the western tussock moth larvae. Approximately 15% of the larvae had pupated at the time of application of DDT, however, and these pupae continued to develop and to transform into moths which mated and deposited eggs which hatched and provided a population level approxi-

Table 1.—List of parasite, hyperparasite, and predator species attacking *Hemerocampa vetusta* (Bdv.).

ORDER	FAMILY	GENUS AND SPECIES	REMARKS
Hymenoptera	Ichneumonidae	*Iseropus orgyiae* (Ashm.)	Most common. Several parasite adults emerged from each prepupal larva
Hymenoptera	Ichneumonidae	*Coccygomimus sanguinipes* (Cress.)	Common. Large parasitic species which emerged singly from pupa
Hymenoptera	Ichneumonidae	*Phobocampe* sp.	Probably new. Common. Parasite emerged from larger larvae
Hymenoptera	Braconidae	*Bracon xanthonotus* Ashm.	Common. Small parasitic species emerged from pupa
Hymenoptera	Pteromalidae	*Dibrachys cavus* (Walk.)	Common. Hyperparasite of *Phobocampe* sp.
Hymenoptera	Pteromalidae	*Brachymeria ovata abiesae* (Gir.)	Not common. Pupal parasite
Diptera	Larvaevoridae	"*Phorocera*" *fuscimacula* Ald. & Webb.	Common larval parasite
Coleoptera	Dermestidae	*Trogoderma sternale* Jayne var.	Common. Larvae feed on eggs in egg masses as a predator
Hemiptera	Anthocoridae	*Lyctocoris campestris* (F.)	Not common. Observed feeding on pupa as predator

mately as high as that in the experimental grove during the spring of 1956. Therefore, the DDT treatment was applied too late to reduce tree damage, but lowered the population carryover for 1957 by 85%.

Results of experimental chemical control studies made in 1957 to determine the efficacy of various pesticides used to control the larvae of the western tussock moth on navel and Valencia oranges are presented in table 2.

Larval counts to determine the population levels in the citrus plots and the efficacy of the pesticide treatments were made on a per-hour-search basis (Atkins *et al.* 1957). For example, if 20 living larvae are found in 10 minutes, the total larval population would be 120 caterpillars on a per-hour-search basis. In the tests reported herein a uniform period of time was used for each treatment and replicate.

The general criteria used to indicate whether a material is efficacious as a pesticide to control this pest is that the material must be capable of reducing the larval population a minimum of 95% within a period of 1 week after it application. The cost of the pesticide and the presence or absence of unfavorable side effects on other pest insects and mites present in the grove are other considerations to be weighed before a material may be recommended for use as a control measure.

Of the effective pestisides listed, three, namely, parathion, TDE, and DDT, are registered for use on citrus and will provide very satisfactory control when applied as complete foliage sprays at the rate of 2, 5, and 5 pounds, respectively, of the toxicant per acre. Dilan, Guthion, methyl parathion, Phosdrin, and Sevin (N-methyl-1-naphthyl carbamate) were effective controls but they are not registered for use on citrus at present and therefore cannot be utilized. The remaining materials were not effective pesticides at the dosages used in these tests.

Cryolite was being used extensively as a commercial treatment in the Corona area and was therefore tested in the second series of applications. In experimental tests, cryolite was inadequate as a control measure. Malathion, which is in general use on citrus to control California red scale, aphids, and mites, was not effective as a control for the larvae of the western tussock moth. Observations during 1956 and 1957 indicate that malathion, when used at certain times of the year for control of California red scale (*Aonidiella aurantii* (Mask.)), causes more severe infestations of the western tussock moth by killing off its egg predator *Trogoderma sternale* Jayne and thereby allowing more eggs to survive (Atkins 1957b).

Table 2.—Efficacy of various pesticides used to control larvae of *Hemerocampa vetusta* (Bdv.), on navel and Valencia orange trees at West Covina, California (J. B. Hytower Grove).[a]

FORMULATION[b]	POUNDS TOXICANT PER ACRE	AFTER 1 WEEK	
		No. of Larvae[c]	Per Cent Larval Reduction
Trees sprayed April 16, 1957			
Parathion, 25% EC	1	0	100
Methyl parathion, 74.2% EC	1	0	100
Dilan, 25% EC	4	0	100
TDE (DDD), 25% EC	3	2	99
Sevin, 24% EC	1.5	6	98
DDT, 25% EC	3	24	92
Guthion, 18.4% EC	1	36	88
Hercules AC-528, 25% EC	2	40	87
Diazinon, 25% EC	1.5	48	84
Dicaphthon, 25% EC	1	105	65
Dipterex, 50% sol. pwdr.	1.5	120	60
Malathion, 57% EC	2	216	28
Chlorthion, 25% EC	1.5	240	20
Untreated	—	300	—
Trithion, 37% EC	2	444	−48
L.S.D. at 5% level		13.56	
at 1% level		18.11	
Trees sprayed April 30, 1957			
Parathion, 25% EC	2	0	100
TDE (DDD), 25% EC	5	0	100
DDT, 25% EC	5	0	100
Dilan, 25% EC	5	0	100
Guthion, 23.9% EC	2	0	100
Methyl parathion, 80% EC	1.5	0	100
Phosdrin, 25% EC	2	0	100
Cryolite, 70% pwdr.	50	36	69
Dicaphthon, 25% EC	2	45	61
Cryolite, 90% pwdr.	48	48	58
Toxaphene, 8 lbs./gal. EC	6	48	58
Untreated	—	115	—
L.S.D. at 5% level		6.21	
at 1% level		8.34	

[a] Six-tree replicates treated with CES Entomology 100-gal. sprayer at 500 p.s.i., using conventional hand guns giving a complete foliage spray (approximately 15 gal. of spray per tree). Larvae were mostly one-third to one-half grown at time of application.

[b] EC = emulsifiable concentrate; sol. pwdr. = soluble powder; pwdr. = powder.

[c] Number of larvae per-hour-search basis; average of four replicate counts for each treatment.

REFERENCES CITED

Atkins, E. L., Jr. 1957a. Western tussock moth. California Citrog. **42**(6): 227–8.

Atkins, E. L., Jr. 1957b. Biology and control of the so-called

"Orangeworms" attacking citrus. 1957 Ann. Progress Rept. of Project No. 1084, pp. 150–60.

Atkins, E. L., Jr., *et al.* 1957. The omnivorous leaf roller, *Platynota stultana* Wlshm., on cotton in southern California: Damage and Control. Jour. Econ. Ent. 50(1): 59–64. (See "Methods," p. 60.)

Forbes, A. G. 1957. California Dept. Agric., Bur. Ent. Cooperative Insect Pest Report, May, 1957, pp. 1–5. (See p. 2.)

Osborn, H. T. 1956. California Dept. Agric., Bur. Ent. Cooperative Insect Pest Report, May, 1956, pp. 1–5. (See pp. 2–3.)

Volck, W. H. 1907. The California tussock moth. Univ. California Expt. Sta. Bull. 183: 189–216.

The Residual Effect of Insecticides Applied to Meadow and Pasture Sod for Control of the European Chafer[1]

H. H. SHOREY, W. G. EVANS,[2] R. H. BURRAGE,[3] and GEORGE G. GYRISCO,[4] *Cornell University, Ithaca, N. Y.*

ABSTRACT

The soil in the plots of each of five experiments established between 1950 and 1954 was sampled at intervals ranging from 1 to 3 years to determine the residual effectiveness of various insecticides upon larvae of the European chafer, *Amphimallon majalis* (Raz.). Parathion at 8 pounds of actual toxicant and BHC at 8 pounds of gamma isomer per acre had lost their effectiveness by 3 and 6 years, respectively, after application, while chlordane at 8 pounds was still providing almost complete control after 7 years. Strobane at 5 or 10 pounds and toxaphene at 10 or 20 pounds per acre gave inferior control during the year of application and succeeding years. The remainder of the materials, aldrin, dieldrin, endrin, isodrin, and heptachlor, showed evidence of a lasting residual effectiveness at rates of application varying between 0.5 and 2 pounds of actual toxicant per acre.

Several insecticides are known to afford effective control of larvae of the European chafer, *Amphimallon majalis* (Raz.), which often cause extensive damage to meadows and pastures by feeding on the roots of grasses and fibrous-rooted forage legumes. These materials include aldrin, dieldrin, and heptachlor at 2 pounds and chlordane at 10 pounds of actual toxicant per acre, all of which have given consistently excellent results during the year of application in experiments conducted under a variety of conditions. A high degree of control has been obtained at rates often considerably lower than those mentioned above, which are the amounts recommended to New York growers.

Inasmuch as many of the meadows and pastures within the area of European chafer infestation in New York are of marginal value, and because larval densities and the severity of the damage fluctuate from year to year, most growers are unwilling to make continuous yearly applications of insecticides. Therefore, a desirable characteristic for an insecticide used for control of the European chafer in meadow and pasture sod is its ability to persist and remain effective for several years.

In each of the experiments to be discussed, the various insecticides were applied to the soil surface in plots arranged in a randomized complete block design with four adjoining replications. During October or November of the year of application, 1-square-foot holes were dug to a depth of approximately 8 inches in four (5 in 1950) randomly selected locations in each plot. The soil removed was hand-sorted for a determination of the number of larvae present. The same sampling procedure was repeated at intervals ranging from 1 to 3 years during the entire test period in an attempt to evaluate the residual effectiveness of the materials used. Results obtained from the samplings for each experiment were combined for statistical analysis. This procedure allowed comparisons among the degrees of larval control provided by the various treatments averaged over the years as well as within each year.

EXPERIMENT 1.—Chlordane and parathion, each at the rate of 8 pounds of actual toxicant per acre, and BHC at 8 pounds of gamma isomer per acre were applied as dusts in a permanent pasture located in Port Gibson, New York on April 8, 1950. Burrage & Gyrisco (1953) described the details of formulation and application and presented the results obtained in a series of larval samplings conducted during the first 2 years following application. Average numbers of larvae per square foot found in each of the treatments during additional samplings conducted on October 9, 1953, November 9, 1956, and October 31, 1957 are presented with the numbers found on October 20, 1950 in table 1.

EXPERIMENT 2.—Granulated formulations of aldrin, dieldrin, and heptachlor at 1 and 2 pounds, chlordane at 10 pounds, and toxaphene at 10 and 20 pounds of actual toxicant per acre were applied to a meadow in Newark, New York on August 6, 1953. Details of application, results of samplings conducted during the first 2 years, residue data, and data on increased hay yields resulting from European chafer control were presented by Gyrisco & Evans (1954) and Evans & Gyrisco (1955). Average numbers of larvae per square foot found in samplings conducted on October 25, 1955 and November 2, 6, and 9, 1956 are included with those reported by the above authors for 1953 and 1954 in table 1.

EXPERIMENT 3.—Granulated formulations of endrin at 0.5, 1, and 2 pounds, dieldrin at 0.5 and 1 pound, and Strobane at 5 and 10 pounds of actual toxicant per acre were applied to an abandoned pasture located in Marion, New York on August 2, 1954. The required amount of

[1] Accepted for publication April 21, 1958.

[2] Present address: Department of Entomology, University of Alberta, Edmonton, Alberta, Canada.

[3] Now Entomologist at the Dominion Entomological Laboratory, Saskatoon, Saskatchewan.

[4] The authors thank D. G. Harcourt, Lemac Hopkins, E. W. Huddleston C. S. Koehler, D. S. Marshall, A. A. Muka, H. H. Neunzig, and R. L. Ridgway who aided in parts of the field work, and H. H. Schwardt, who edited the manuscript.

Table 1.—The residual effect of several insecticides upon populations of the European chafer in meadow and pasture sod.

Insecticide	Actual Toxicant (Lbs./A.)	Mean Number[a] of Larvae per Square Foot After Specified Years						Mean
		¼ to ½	1 to 1½	2 to 2½	3 to 3½	6 to 6½	7 to 7½	
Experiment 1 (dusts applied April 8, 1950)								
Chlordane	8	0.1			0.0	0.2	0.1	0.1
BHC	8	0.1			0.2	3.2	2.0	1.1
Parathion	8	0.5			2.5	5.0	2.2	2.4
Untreated	—	15.3			1.9	2.8	2.3	4.6
Experiment 2 (granules applied August 6, 1953)								
Dieldrin	2	1.0	0.0	0.1	0.0			0.2
Dieldrin	1	1.3	0.0	0.0	0.1			0.3
Aldrin	2	1.9	0.1	0.8	0.0			0.6
Heptachlor	2	2.7	0.3	1.3	0.4			1.0
Chlordane	10	3.3	0.4	1.8	0.4			1.3
Aldrin	1	3.2	1.2	2.9	0.2			1.7
Heptachlor	1	3.8	1.3	3.5	1.4			2.4
Toxaphene	20	10.7	1.4	3.6	0.1			3.0
Toxaphene	10	12.5	2.1	6.0	2.3			5.1
Untreated	—	23.3	4.6	11.3	2.4			8.9
Experiment 3 (granules applied August 2, 1954)								
Endrin	2	0.2	0.1					0.2
Dieldrin	1	0.7	0.2					0.4
Endrin	1	1.1	0.2					0.6
Endrin	0.5	0.9	1.1					1.0
Dieldrin	0.5	1.2	0.9					1.1
Strobane	10	2.9	2.6					2.7
Strobane	5	3.0	4.9					3.9
Untreated	—	4.0	4.3					4.1
Experiment 4 (granules applied August 2, 1954)								
Isodrin	2	0.0			0.1			0.1
Isodrin	1	0.3			0.1			0.2
Aldrin	1	0.3			0.2			0.3
Aldrin	0.5	0.5			0.2			0.4
Heptachlor	0.5	0.7			0.6			0.6
Heptachlor	1	0.7			0.6			0.6
Isodrin	0.5	1.2			0.3			0.8
Untreated	—	3.7			0.8			2.1

Any two means adjacent to the same solid line are not significantly different. Any two means within the same column adjacent to dotted lines are not significantly different. Significance determined by Tukey's honestly significant difference test at 5%.

[a] Data were transformed for statistical analysis: $X' = \sqrt{\bar{x}} + 0.5$ where $\bar{x}$ was the mean number of larvae found in each plot. Means presented in the right-hand column are not exact arithmetic averages of other means in the table, but were calculated as means of the transformed data and were then converted back to numbers of larvae.

material for each 1/100-acre plot was brought to a weight of 8 pounds by the addition of pyrophyllite and applied with a hand-operated fertilizer spreader. Results of larval samplings conducted on October 19 and 24, 1954 and October 25, 1955 are included in table.1

Experiment 4.—Granulated formulations of aldrin and heptachlor at 0.5 and 1 pound and isodrin at 0.5, 1, and 2 pounds of actual toxicant per acre were applied to a pasture located in Marion, New York on August 2, 1954. The application data are identical to those described for Experiment 3. Table 1 contains the results of larval samplings, which were conducted on October 6 and 11, 1954 and October 19, 1957.

Experiment 5.—Evans & Gyrisco (1955) applied granulated dieldrin to a meadow in Sodus, New York on August 7, 1954 by means of several hand methods which are available to many growers. Application was effected at the rates of 1 and 2 pounds of actual toxicant per acre with a cyclone seeder, and at 2 pounds per acre with a hand-operated fertilizer spreader and a wheelbarrow seeder. These methods were compared with a commonly used standard consisting of an application of dieldrin at 2 pounds of actual toxicant per acre as an emulsion by means of a low-volume, low-pressure weed sprayer. Larval samplings conducted on October 2, 1954 revealed that all of the treatments had reduced larval populations by at least 90%. When the plots were again sampled on October 26, 1957, no larvae were found in any of the treated plots, though a total of 46 larvae was found in the samples taken from the four untreated check plots.

Discussion.—Because of the long residual life of many of the materials tested and the variability in larval counts, only the most distinct trends were detected in loss of effectiveness as measured by European chafer control. By the end of a 7-year period the reduction in the residual effectiveness of BHC and parathion was clearly apparent (Experiment 1). Parathion at 8 pounds per acre had lost all capacity to reduce larval populations by 3 years after application. BHC at 8 pounds of gamma isomer was giving excellent control after 3 years, but had lost its effectiveness by 6 years after application, while chlordane at 8 pounds continued to afford almost complete control more than 7 years following application.

Strobane at 5 and 10 pounds per acre (Experiment 3) and toxaphene at 10 and 20 pounds per acre (Experiment 2) did not afford sufficient control during the first year or in succeeding years to be of further interest. The other materials, aldrin, dieldrin, endrin, isodrin, and heptachlor, showed evidence of a lasting residual effectiveness in that, although they did not all kill the same number of larvae, the degree of control which each provided remained approximately constant during test periods ranging from 1.5 to 3.5 years.

No significant differences were detected among any of the treatments and the check in Experiment 4 as a result of the sampling conducted 3 years following application. The lack of apparent differences probably may be attributed more to the very low larval densities found in the experimental area during that year than to a loss in effectiveness of the insecticides.

Often it is noticed in European chafer control experimentation that the reduction of larval populations provided by the most effective materials during the year of their application is not as great as during succeeding years. This delay in effectiveness is indicated to some extent in Experiments 2, 3, and 5. In many of such cases application was effected during the summer, when the larvae were in the first and second instars, and the first larval sampling was conducted during October or November of the same year. Depending on such factors as seasonal precipitation, porosity of the soil, and solubility of the insecticide, a toxicant applied during the summer may not penetrate to a sufficient depth in the soil until larvae are in a more advanced stage of development. During succeeding years and succeeding generations, however, newly hatched first instar larvae, as well as the ovipositing adults, are exposed to the toxicant if it is well dispersed through the upper layers of the soil and has a long residual effectiveness. Gambrell (1953) has shown that first instar European chafer larvae are more susceptible to DDT than second instar larvae, and that both the first and second instars are considerably more susceptible than third instar larvae, and Shorey & Gyrisco (1958) demonstrated that as much as 50% of the initial reduction of larval populations in dieldrin-treated soil could be due to the effect of the insecticide upon the adults.

REFERENCES CITED

Burrage, Robert H., and George G. Gyrisco. 1953. Control of European chafer damage in pasture sod—II. Jour. Econ. Ent. **46**(1): 142–6.

Evans, William G., and George G. Gyrisco. 1955. Granulated insecticides for control of the European chafer in meadows. Jour. Econ. Ent. **48**(4): 384–6.

Gambrell, F. L. 1953. Control of the European chafer, *Amphimallon majalis* Raz., in turf. Jour. Econ. Ent. **46**(5): 761–5.

Gyrisco, George G., and W. G. Evans. 1954. Granular insecticides. Agric. Chemicals 9(1): 32–33.

Shorey, H. H., and George G. Gyrisco. 1958. The effect of entry into dieldrin-treated soil upon adults of the European chafer. Jour. Econ. Ent. **51**(4): 486–8.

A Study of Soil-Inhabiting Mites from Connecticut Apple Orchards[1]

HENRY W. HURLBUTT, *Department of Zoology, University of Maryland, College Park*

ABSTRACT

The sod from five Connecticut apple orchards was sampled at 12- to 16-day intervals for a period ranging from 8 months to 1 year, and the mite fauna in each orchard was analyzed. The orchards included an abandoned orchard, a lightly sprayed orchard, and three heavily sprayed orchards. In the first two orchards a relatively large number of predatory species occurred with no one species making up an excessively large portion of the total number of individuals. In the three heavily sprayed orchards the number of species was smaller, and the great majority of individuals belonged to only one or two species. *Leioseius minusculus* Berlese was very abundant in all three heavily sprayed orchards, although it was also present in the other two orchards sampled. Tetranychidae were also most numerous in the heavily sprayed plots.

In one of them a pronounced rise in numbers of two species of Parasitoidea, *Parasitus americanus* Berlese and *L. minusculus*, was observed following the application of mulch. It is believed that the increase of *P. americanus* was due to an increased supply of Collembola and Acaridiae.

Tetranychidae (spider mites) are seldom important pests of apple trees in unsprayed orchards. Following the use of insecticides, however, these mites frequently become very numerous and do severe damage to the foliage. Several reasons have been suggested for this phenomenon, one of which is that the insecticides destroy predators of the Tetranychidae. As a result, a number of studies of predatory mites and insects occurring upon the foliage and bark of apple trees have been made (Collyer 1956, 1953, Pickett & Patterson 1953, Clancy & Pollard 1952, Lord 1949, Garman 1948). Predatory species living in the sod under the trees have received much less attention despite the fact that two of the tetranychid pests of apple, *Tetranychus bimaculatus* Harvey and *Bryobia praetiosa* Koch, spend part of their life cycles on the ground. Dr. Philip Garman of the Connecticut Agricultural Experiment Station suggested that some of the mites living in the sod may act as predators of spider mites. Acting upon this suggestion an investigation of the mite fauna of orchard sod in Connecticut was undertaken.

The object of the present study was to ascertain if there are differences in the number, kind and variety of predators occurring in sprayed and unsprayed apple orchards. Ultimately it was hoped that the study might determine whether or not soil inhabiting mites play a role in controlling spider mites. Sheals (1956, 1955) showed that populations of Collembola actually increased following applica-

[1] Part of a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Connecticut. Accepted for publication May 8, 1958.

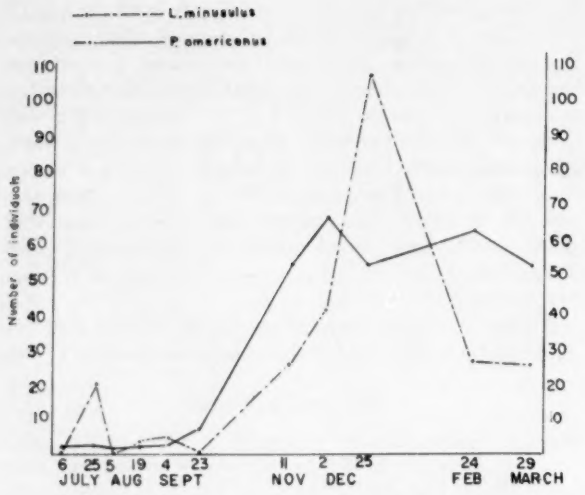


FIG. 1.—*Leioseius minusculus* (Berlese) and *Parasitus americanus* (Berlese) in a heavily sprayed orchard, Middlefield, Connecticut, 1956–57. Mulch was added in early September.

tion of DDT, and attributed this increase to a reduction in numbers of predatory Mesostigmata. It was thought that possibly the large numbers of the Tetranychidae in sprayed orchards might also be due in part to destruction of predatory soil-inhabiting Acarina by insecticides.

METHODS.—Five orchards were selected and sampled at approximately 14-day intervals (less often during the winter) for periods ranging from 8 months to a year during 1956 and 1957. Six chunks of sod, each from under a different tree and totaling 110 square inches in surface, were taken from each orchard on each collection date. Although the use of the same six trees probably resulted in a less complete picture of the orchard it was felt that this procedure would reduce the sample variation caused merely by selection of different trees. The samples were usually between 1 and 3 inches (2.5 to 7.5 cm.) deep. They were taken at random distances from the base of the tree but were always within the circumference of the outermost branches. The sod was brought back to the laboratory in paper bags, placed upside down in Tullgren funnels, and left in the funnels until no more mites were recovered. The practice of placing the samples upside down is recommended by Macfadyen (1955) and Hammer (1934) to insure that surface-living animals will not burrow for long distances while those that live deeper, and can burrow, will do so.

The mites were collected in bottle of 70% alcohol screwed into the bottoms of the funnels. Most specimens were counted by examination with a dissecting microscope. A portion of the recognized species and all individuals whose identity was questionable were mounted in Hoyer's solution for identification with the aid of a compound microscope. The Oribatei, none of which are known to be predators, were treated as a unit. A distinction was made, however, between adults and nymphs. The number of oribatid nymphs collected was very low throughout the course of the study. The weakness of Berlese apparatus in extracting larvae and nymphs of Oribatei has been pointed out by Jacot (1936). The Trombidiformes were first identified to family under the dissecting microscope.

Table 1. Number of species of Mesostigmata and Trombidiformes collected from each of five orchards.

ORCHARD	NO.[a]	MESOSTIGMATA	TROMBIDIFORMES
Somers, abandoned	18	21 (4)[b]	13 (1)
Houston, lightly sprayed	18	16 (3)	10 (1)
Middlefield, heavily sprayed	11	12 (4)	5 (3)
University, heavily sprayed	11	9 (2)	4
South Windsor, heavily sprayed	16	7 (2)	3

[a] No. = number of collecting dates.
[b] Figures in parenthesis indicate the number of species occurring on single occasions.

A portion of these were then mounted on slides for future identification. In the case of the Mesostigmata, at first all specimens were mounted, but soon it was possible to recognize most species with the use of only the dissecting microscope.

A series of specimens representing all species reported in this paper has been deposited in the collection of the Connecticut Agricultural Experiment Station at New Haven and a representative collection has been placed in the collection of the Department of Zoology and Entomology of the University of Connecticut, Storrs.

Abandoned orchard, Somers.—The orchard at Somers had not been used on a commercial basis for about 6 years previous to this study. The trees were from 20 to 25 years old. The orchard was on a hillside and was bounded on the west by woods. Underneath the trees the vegetation consisted mostly of grass. There were also numerous clumps of goldenrod, *Solidago* spp., and scattered clumps of the sensitive fern, *Onoclea sensiblis* L., in the orchard, but the samples were all from areas covered with grass. Fallen apple leaves and the dead stems of the previous years growth of grass littered the sod. Both earthworms and isopods were present and the castings of the former were evident.

Lightly sprayed Houston's orchard, Storrs.—The plot sampled from this orchard received lead arsenate at 3 lbs./100 gal. and fermate at 1½ lbs./100 gal. about every 10 days during the 1956 season. No other chemicals were applied except for a small amount of sulfur at the beginning of the season and one light dosage of methoxychlor. In 1955 the orchard was not sprayed at all after the pink stage.

Straw, small twigs, dead oak and apple leaves, and rotting apples littered the ground. The soil was very loose and crumbly. The vegetation under the trees sampled consisted mostly of orchard grass, which was not cut until late in the summer. Clover was absent under all the trees sampled, although scattered clumps were present between the rows.

Heavily sprayed orchards.—The plot from South Windsor was mowed frequently and had been plowed about 6

Table 2. Average number of individuals per collecting date for each of the major groups of mites recorded.

ORCHARD	PARASITOIDEA	UROPODOIDEA	PREDACEOUS TROMBIDIFORMES	TETRANYCHIDAE	ORIBATEI (ADULTS)
Somers	71.7		21.6	2.8	357.7
Houston	62.8	9.6	12.9	3.7	51.3
Middlefield	56.8		.4	10.2	480.8
University	54.0		.4	36.9	398.3
South Windsor	23.5		3.6	22.8	36.7

years prior to the present study. In the plot studied the cover consisted of scattered clumps of grass and some clover. The soil below the grass roots was rather stony and around the base of each tree was a circle of ground which was almost completely bare early in the season but had some moss and everlasting, *Anaphalis margaritacea* (L.), growing on it later in the season. There was little litter or mulch anywhere.

Insecticides were applied to the plot every week or two from June 2 until August 15. A single application of dieldrin at one-half pound per 100 gallons of water was applied June 2. Other insecticides used were methoxychlor, lead arsenate, malathion, DDD and DDT, all at concentrations of 2 pounds per 100 gallons of water. Kelthane was applied in late July at 1½ pounds per 100 gallons followed by two applications of Aramite at the same concentration. The fungicides used were sulfur, thiram and captan. The 1955 spray schedule was similar but also included fermate.

The trees in the Middlefield plot were about 30 years old and the ground under them had not been cultivated for at least 20 years. There was grass between the rows, but the ground cover under the trees consisted primarily of Virginia creeper, *Parthenocissus* sp., and poison ivy, *Rhus radicans* L. Where present, these inhibited the growth of other plants and prevented the formation of turf. The soil was loose and crumbly and there was not much litter or dead plant material until early September when hay was piled under the trees.

Insecticides were applied to the Middlefield plot about every 2 weeks from May 28 to August 2. Single applications of parathion and Black Leaf 253 (DDT, 2.5% and parathion, 3%) were put on as a calyx spray. During the remainder of the season the treatments included lead arsenate, DDT and malathion at 2 pounds per 100 gallons of spray, Aramite at 1½ pounds and TEPP. The fungicides used were fermate and captan. All sprays were applied with a Hardy mist sprayer as 4× or 6× concentrates, a procedure which lessens the amount of insecticide running off onto the ground.

The plot from the University orchard was chosen with the intent of finding some correlation between two-spotted spider mite populations and predatory mite populations. This plot had received only methoxychlor in 1955 and the trees were badly burned by two-spotted spider mite damage. During 1956 it received lead arsenate and methoxychlor at 3 pounds, DDT at 2 pounds, sulfur at 6 pounds and 50% dieldrin at ½ pound wettable powder, per 100 gallons of water. In addition to these during late July half the plot received two applications of Kelthane while the other half received two applications of the acaricide, Trithion. Hence the 1956 spray schedule for this orchard was similar to that used in South Windsor, but no malathion, thiram, or captan were used at Storrs.

The cultural practices in the two orchards were also similar but the turf was deeper at Storrs. The plot had not been plowed since about 1940 but had been mowed frequently. The cover consisted of grass and scattered clumps of clover, and around the base of each tree a bare area had been created which was partially covered by grass. Mulch was limited to scattered wisps of dead hay. The trees used for sampling were selected from a smaller area than in any of the other orchards studied, a factor which would be likely to lessen the variety of habitats encountered, and consequently the number of species collected. All trees in the plot were of the McIntosh variety.

Table 3.—Number of mites in sod samples from Middlefield, a heavily sprayed orchard. 1956–57.

Date	Oribatei (Adults)	*Bryobia* sp.	*P. americanus*	Phytoseiidae Spp.	*L. minusculus*	Total Parasitoidea
July 6	175	0	2	0	0	2
July 25	530	0	2	0	19	22
Aug. 5	180	0	1	0	0	3
Aug. 19	85	0	2	0	3	9
Sept. 4[a]	591	0	2	3	4	12
Sept. 23	380	7	7	3	1	14
Nov. 11	710	4	53	5	25	87
Dec. 2	600	10	67	0	40	111
Dec. 25	1070	6	53	8	107	177
Feb. 24	394	15	63	3	26	108
March 29	574	66	53	2	25	80
Total	5289	108	305	24	250	625
Average	480.8	9.8	27.7	2.2	22.7	56.8

[a] Mulch was placed under the trees in early September.

RESULTS.—Some idea of the greater variety of Acarina occurring in the abandoned and lightly sprayed plots may be gained by comparing the number of species of Mesostigmata and Trombidiformes from each orchard (table 1). The number of individuals of predaceous species occurring in these orchards was also somewhat greater than in the heavily sprayed orchards (table 2). The phytophagous Tetranychidae were more abundant in the heavily sprayed orchards. However, the values given in table 2 for spider mites are subject to considerable error due to the large seasonal fluctuations exhibited by these mites. In the case of the suborder Sarcoptiformes, figures are given only for adult Oribatei. Immature Oribatei were very poorly extracted by the method used, while Acaridiae are not included, because during the first part of the study the author was unable to distinguish them from oribatid nymphs.

Closer examination of the data on the Parasitoidea shows that in only one of the three heavily sprayed orchards, the South Windsor plot, was the average number of Parasitoidea greatly less than in the abandoned Somers plot or the lightly sprayed Houston's orchard. However, in Somers and Houston's orchard the individuals were scattered among a number of different species, while in the three heavily sprayed orchards one or two species made up a vast majority of the total. For instance, in Somers the most abundant species of Parasitoidea, *Pergamasus* sp., made up only 24.5% of the total number

Table 4.—Number of mites in sod samples from the University orchard, a heavily sprayed orchard. 1956.

Date	Oribatei (Adults)	Tetranychidae	Phytoseiidae	*L. minusculus*	*Hypoaspis* sp.	Total Parasitoidea
April 28	411	17	0	56	0	59
May 12	630	29	2	30	0	33
May 24	287	50	0	14	0	14
June 9	290	84	3	12	0	15
June 24	107	80	16	11	0	29
Aug. 4, 11[a]	385	2	0	191	0	201
Aug. 18	452	30	2	36	25	77
Sept. 3	393	5	0	5	12	22
Oct. 3	521	18	0	15	2	27
Oct. 20, 24[a]	477	67	3	27	30	63
Nov. 9	428	24	0	18	35	54
Total	4381	406	26	415	104	594
Average	398.3	36.9	2.4	37.7	9.5	54.0

[a] One-half of sample taken on each date.

Table 5.—Number of mites in sod samples from South Windsor, a heavily sprayed orchard. 1956–57.

Date	Oribatei (Adults)	Tetranychidae	*Ledermuelleria* n. sp.[a]	*Lasioseius penicilliger*[b]	*Leioseius minusculus*	Total Parasitoidea
April 14	9	12	1	0	20	21
April 28	45	46	6	0	17	17
May 26	89	14	2	0	6	7
June 23	47	0	6	1	12	20
July 7	29	1	11	1	21	23
July 22	115	0	13	6	14	31
Aug. 4	46	0	1	48	37	89
Aug. 18	20	0	0	0	1	6
Aug. 30	26	0	0	0	38	39
Sept. 14	37	8	0	2	20	24
Sept. 30	39	15	0	1	48	52
Oct. 20	13	35	0	0	14	14
Nov. 10	15	72	0	0	12	15
Nov. 24	29	60	0	0	2	2
Dec. 31	18	15	8	0	6	8
March 23	11	86	10	1	5	7
Total	588	364	58	60	273	375
Average	36.7	22.8	3.6	3.7	17.1	23.5

[a] Determination by Dr. F. M. Summers, University of California, Davis.
[b] Determination by Dr. G. O. Evans, British Museum of Natural History.

of individuals while two species of *Veigaia*, *V. nemorensis* (Koch)[2] and *V. cerva* (Kramer), together constituted 20.9%. The third most abundant species of parasitoid in Somers, *Digamasellus angulosus* Willmann, made up 12.9% of the total number of Parasitoidea. In Houston's orchard 35.0% of the Parasitoidea collected belonged to a single species, *Veigaia nemorensis* (Koch) and 19.2% belonged to two species of *Asca*, *sphidioides* (L.) and *bicornis* (Can. & Fanz.). The third most abundant parasitoid here, *Hypoaspis* sp., constituted 15.4% of the total.

In the heavily sprayed University orchard, on the other hand, a single species, *Leioseius minusculus* Berlese,[3] comprised 70.0% of the total number of Parasitoidea while 17.5% belonged to *Hypoaspis* sp. *L. minusculus* was also the most abundant parasitoid in South Windsor, where it constituted 72.4% of the parasitoid population. In Middlefield two species, *Parasitus americanus* Berlese[4] with 48.8% and *L. minusculus* with 40.0% made up almost nine-tenths of the predator population.

Several explanations for the less varied fauna of predatory mites in the heavily sprayed orchards suggest themselves. First there is the obvious suggestion that insecticides eliminate or reduce certain species. At South Windsor it may be that the lack of much humus prevented some species from surviving there. The pronounced rise in numbers of *Parasitus americanus* and *Leioseius minusculus* following application of mulch to the Middlefield plot indicates the value of decaying vegetation even for predatory species. Probably the beneficial effects of the mulch were due primarily to the fact that it provided more food for Collembola and Acaridiae, which in turn, served as prey for predatory species. In Somers and Houston's orchard the presence of litter may have provided a suitable habitat for some of those species which were absent in the heavily sprayed orchards. The relatively large numbers of earthworms in Somers may have been partially responsible for the varied fauna found there. According to Weis-Fogh (1948), the tables of Frenzel (1936) show that in localities where the earthworm fauna is abundant the greatest microfauna exists, and that in localities with few earthworms the microfauna below the surface is poor.

Collyer (1953) in a study comparing mite and insect faunas of commercial and neglected orchards in southeastern England also found a more varied fauna in neglected orchards. She observed that in neglected orchards many different species occur but none in excessive numbers. In commercial orchards, on the other hand, she found that the fauna consists of relatively few species, although the number of individuals may be very high, and populations are subject to violent fluctuations.

It is impossible to make generalizations about the fauna of heavily sprayed orchards as contrasted with unsprayed or lightly sprayed orchards on the basis of only five orchards. Nevertheless some comparisons may be made. *Leioseius minusculus* was very abundant in all three of the heavily sprayed orchards. It was present in the lightly sprayed Houston's orchard, but at a low density, and in Somers it was collected only twice.

Parasitus americanus, a large and active mite belonging to the family Parasitidae, was present in all three of the heavily sprayed orchards but was never collected from Somers or Houston's orchard. In the latter two orchards another large parasitid species, *Pergamasus crassipes* var. *longicornis* Berlese, was present. Members of the genus *Veigaia* were very abundant in both Somers and Houston's orchard, but were totally absent in the three heavily sprayed orchards. It may be that the dead leaves and twigs in Somers and Houston's orchard were in some way responsible for the abundance of *Veigaia*, perhaps through creation of a suitable habitat for Collembola. According to Sheals (1956) *Veigaia nemorensis* preys upon Collembola.

As was expected Tetranychidae were more abundant in the heavily sprayed orchards. Most of the spider mites collected from sod were *Bryobia*, or clover mites. In late autumn these were observed congregating in large numbers under bark of trees in the South Windsor and University orchard plots. Phytoseiidae, a family of mites whose members often feed upon tetranychids, were equally abundant in all of the plots except South Windsor where only two individuals were collected.

Oribatei were most abundant in Middlefield, the University orchard, and Somers. This group was much less numerous in Houston's orchard and South Windsor. In the case of South Windsor the heavy spray schedule and shallow turf might account for the small numbers of Oribatei.

Collembola, which serve as food for many Parasitoidea, were very abundant in Middlefield and Houston's orchard, fairly numerous in Somers, and relatively scarce in South Windsor and the University orchard. In the abandoned Somers orchard thrips, Staphylinidae, pseudoscorpions and a considerable variety of other insects and arachnids were present in the sod. In the sod of the heavily sprayed orchards predaceous insects were virtually absent.

Seasonal variation.—Because the number of samples used was small and the method of extraction imperfect, apparent seasonal fluctuations in population density must be treated with caution. For one thing, the depth of the samples was not constant. When the ground was wet, dirt tended to cling to the roots whereas when the sod was dry some soil tended to be shaken loose from the roots.

[2] Determination by Dr. M. H. Farrier, North Carolina State College.
[3] Determination by Dr. G. O. Evans, British Museum of Natural History.
[4] The identity of this species is questionable.

Table 6.—Number of mites in sod samples from a lightly sprayed orchard, Houston's orchard, Storrs. 1956–57[a]

DATE	ORIB.	UR.	TR.	O.P.	V.N.	PHYT.	*Asca* SPP.	HYPO. SP.	N.	PAR.
April 13	23	2	4	1	14	0	0	3	0	19
April 27	53	0	6	1	4	0	0	1	0	6
May 11	64	0	3	2	2	0	0	4	0	8
May 25	78	2	5	3	13	0	0	1	0	17
June 9	149	10	2	2	36	0	1	4	0	45
June 24	37	10	1	1	10	0	4	3	12	30
July 7	32	6	1	0	20	1	3	5	21	50
July 22	61	22	15	10	39	0	3	9	30	99
Aug. 4	33	13	25	2	13	4	15	6	25	71
Aug. 18	75	8	7	0	5	4	7	9	20	48
Sept. 3	56	9	1	1	43	0	11	42	18	116
Sept. 16	96	11	8	0	31	3	20	24	9	90
Sept. 30	44	26	16	9	34	11	47	19	0	138
Oct. 20	24	7	12	1	14	2	29	9	0	62
Nov. 12	46	3	19	3	52	8	23	21	0	121
Nov. 25	80	25	25	13	16	5	23	7	0	82
Dec. 31	43	18	12	11	26	4	18	6	0	84
March 16	40	1	2	3	25	1	14	2	0	49
Total	924	173	164	63	397	43	218	175	135	1115
Average	51.3	9.6	9.1	3.5	22.1	2.4	12.1	9.7	7.5	62.8

[a] Only the most frequently encountered species are included in the table.
Abbreviations used: Orib., Oribatei (adults only); Ur., Uropodina; Tr., Trombidiidae; O.p., *Ololaelaps placentula* (Berlese); V.n., *Veigaia nemorensis* (Koch); Phyt., Phytoseiidae; Hypo. sp., *Hypoaspis* sp.; N., parasitoid nymphs which could not be identified at the time, but most of which probably were *Veigaia nemorensis;* Par., total number of Parasitoidea.

Even if the depth of the samples had not varied, there is the possibility that some mites may migrate to deeper soil in dry weather. According to Jacot (1936), Oribatei orient themselves vertically according to moisture. Also the irregular occurrence of some species suggests that these species occur in local aggregations.

In both Somers and Houston's orchard Parasitoidea were lowest in numbers during the spring of 1956. From midsummer until the end of November Parasitoidea were considerably more numerous in these two orchards while rather low numbers of this group were collected during the single winter collection. A single collection made during the spring of 1957 yielded more Parasitoidea than collections made during the spring of 1956. There is a possibility that the lower numbers of Parasitoidea recovered during the first few collections was due to less efficient extraction during the early part of the study. At the time of the first one or two collections the sod was very wet and water clung to the sides of the funnels, possibly drowning some mites. The numbers of Oribatei were also rather

Table 7.—Number of mites in sod samples from an abandoned orchard, Somers. 1956–57[a]

DATE	ORIB.	CU.	TR.	P.C.	P.SP.	O.H.[b]	*Veig.* SPP.	PHYT.	*Asca* SPP.	D.A.	PAR.
April 12	94	0	4	0	0	1	6	1	0	0	12
April 26	541	0	8	2	0	6	0	3	3	0	15
May 12	160	0	2	1	0	0	0	0	1	11	14
May 25	427	3	1	5	3	2	3	0	2	17	35
June 9	286	0	1	2	6	0	15	1	1	1	37
June 23	271	0	0	2	22	1	2	0	2	1	62
July 7	165	0	1	5	15	6	16	1	3	0	45
July 22	188	1	0	5	12	3	20	0	0	0	56
Aug. 4	275	6	5	19	44	6	13	1	0	1	103
Aug. 18	193	12	3	6	16	4	2	3	14	21	84
Aug. 30	416	17	0	11	55	4	32	2	3	26	172
Sept. 14	509	30	6	4	17	18	12	12	8	1	109
Sept. 30	247	4	3	1	13	4	31	1	10	43	112
Oct. 21	407	4	6	5	38	9	32	3	10	0	106
Nov. 10	953	23	24	4	17	27	22	3	11	7	94
Nov. 24	883	1	50	19	29	13	16	2	0	0	89
Jan. 1	231	16	10	0	7	2	16	4	11	0	41
March 16	193	1	2	5	22	1	32	1	1	38	105
Total	6439	118	126	96	316	101	270	38	80	167	1291
Average	357.7	6.6	7.0	5.3	17.6	5.6	15.0	2.1	4.4	9.3	71.7

[a] Only the most frequently encountered species are included in the table.
[b] Determination by Dr. G. O. Evans, British Museum of Natural History.
Abbreviations used: Orib., Oribatei (adults only); Cu., Cunaxidae; Tr., Trombidiidae; P.c., *Pergamasus crassipes* var. *longicornis* Berlese; P.sp., *Pergamasus* sp.; O.h., *Ololaelaps hemisphaera* Berlese; *Veig.* spp., *Veigaia* spp.; Phyt., Phytoseiidae; D.a., *Digamasellus angulosus* Willmann; Par., total number of Parasitoidea.

irregular. In Somers they were most abundant during November, while in Middlefield they showed somewhat of an increase following the application of mulch.

Effect of mulch.—In one of the heavily sprayed orchards, Middlefield, two species of Parasitoidea, *Parasitus americanus* and *Leioseius minusculus*, increased markedly subsequent to the November collection. The addition of mulch in September and the cessation of spraying in August are thought to be the chief reasons for the increase of these two species commencing in November. Since no comparable rise was observed in the other heavily sprayed orchards following the cessation of spraying, it seems probable that the addition of mulch was the more important factor. Both Acaridiae and Collembola were abundant in the mulch. Since members of the Parasitidae have been reared in the laboratory upon both Acaridiae and Collembola (Sheals 1946, Weis-Fogh 1948), it seems likely that the rise of *P. americanus* was due to an increase of available prey. Since the feeding habits of *L. minusculus* are unknown, an explanation of its increase cannot be offered at the present time.

Two species of Parasitoidea, *Pergamasus crassipes* var. *longicornis* Berlese and *Ololaelaps placentula* Berlese, were kept in modified Huffaker cells and observed to feed upon two-spotted spider mites. However, acceptance of a prey under laboratory conditions does not prove that a predator normally captures such an animal under field conditions, for in the field the prey may be inaccessible to the predator. Acceptance of the importance of Parasitoidea as predators of two-spotted spider mite awaits proof that the predator and prey normally come in contact with each other under natural conditions.

ACKNOWLEDGMENTS.—Sincere thanks are extended to the following: Dr. Philip Garman of the Connecticut Agricultural Experiment Station for suggesting the problem, for aid in identification and for continuous help, advice and encouragement during the entire course of the study; Dr. James A. Slater of the University of Connecticut for counsel and advice throughout the course of the investigation; Mr. William Tunis, Extension Entomologist of the University of Connecticut, for help in selection of orchards to be studied; Mr. Byron Houston of Storrs, Mr. John Lyman of Middlefield, Mr. Joseph Pero of South Windsor, and Mr. Wiley of Somers who generously granted permission to use their orchards and provided information about them; and Dr. G. O. Evans, British Museum of Natural History, Dr. Maurice H. Farrier, North Carolina State College, and Dr. Francis M. Summers, University of California, Davis, for determination of specimens.

REFERENCES CITED

Clancy, D. W., and H. N. Pollard. 1952. Effect of DDT on mite and predator populations in apple orchards. Jour. Econ. Ent. **45**(1): 108–14.

Collyer, E. 1953. Biology of some predatory insects and mites associated with the fruit tree red spider mite (*Metatetranychus ulmi* (Koch) in south-eastern England. IV. The predator-mite relationship. Jour. Hort. Sci. **28**(4): 246–59.

Collyer, E. 1956. Notes on the biology of some predacious mites on fruit trees in south-eastern England. Bull. Ent. Res. **47**(2): 205–14.

Frenzel, G. 1936. Untersuchungen über die Tierwelt des Wiesenbodens. Gustav Fischer, Jena.

Garman, P. 1948. Mite species from apple trees in Connecticut. Connecticut Agric. Expt. Sta. Bull. **520**: 1–27.

Hammer, M. 1944. Studies on the Oribatids and Collemboles of Greenland. Meddel. Grønland **141**: 1–210.

Jacot, A. P. 1936. Soil structure and soil biology. Ecol. **17**: 359–79.

Lord, F. T. 1949. The influence of spray programs on the fauna of apple orchards in Nova Scotia. III. Mites and their predators. Canadian Ent. **86**(8): 202–14.

Macfadyen, A. 1955. A comparison of methods for extracting soil arthropods. *In* Soil Zoology, D. K. McE. Kevan editor. Academic Press, New York, pp. 315–32.

Pickett, H. D., and N. A. Patterson. 1953. The influence of spary programs on the fauna of apple orchards in Nova Scotia. IV. A review. Canadian Ent. **85**: 472–8.

Sheals, J. G. 1955. The effects of DDT and BHC on soil Collembola and Acarina. *In* Soil Zoology, D. K. McE. Kevan editor. Academic Press, New York, pp. 241–52.

Sheals, J. G. 1956. Soil population studies. I. The effects of cultivation and treatment with insecticides. Bull. Ent. Res. **47**(4): 803–22.

Weis-Fogh, Y. 1948. Ecological investigations on mites and collemboles in the soil. Natura Jutlandica **1**: 135–270.

NOTICES TO AUTHORS

Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by Biological Abstracts. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the Bulletin of the Entomological Society of America 3(2): 1. June, 1957.

Authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions (Vol. **49**(4): 575–7) before preparing manuscripts intended for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.

Systemic Insecticides for *Theobroma cacao* L., Their Translocation and Persistence in Foliage and Residues in Cacao Beans[1]

J. S. Bowman[2] and J. E. Casida,[3] *Department of Entomology, University of Wisconsin, Madison*

Abstract

From preliminary screening of 14 systemic compounds with cacao seedlings in Costa Rica, Thimet, Chipman R-6200 (*O,O*-diethyl S-(2-diethylamino)ethyl phosphorothiolate), demeton, Isolan and dimefox were selected for further study. These five systemics were compared on the basis of their translocation and persistence in the foliar parts of mature cacao trees after trunk implantation, their effect on the flavor of chocolate produced from beans from the treated trees, and the level and nature of residues in the beans using radiotracer and anticholinesterase methods of analysis.

Thimet and Chipman R-6200 were readily translocated into the foliar portions of cacao trees and persisted for as long as 20 months after a single implantation treatment. Little or no residues were found in the cacao beans at any time after treatment regardless of the pod position on the trees in relation to the site of implantation. Studies with radioactive R-6200 demonstrated a high concentration of phosphorus-containing residues in the cotyledons of the cacao beans but these materials did not partition into chloroform and did not inhibit cholinesterase and therefore cannot be considered as hazardous residues. No off-flavors were detected in chocolate from beans harvested from any of the systemic treatments but definite off-flavors were obtained when BHC was used as a foliar spray. The insecticidal efficiency of the systemics was not studied because the insect populations in the treated areas were not adequate for this purpose.

In recent years there has been considerable interest in systemic insecticides for the possible control of insects attacking trees. Ripper (1957) has reviewed the literature concerning the efficiency of various systemics and several methods for their application to trees. Hanna and coworkers (1952, 1954a, 1954b, 1955) showed that dimefox (tetramethyl phosphorodiamidic fluoride), when applied to *Theobroma cacao* L. as either a soil drench or by trunk implantation, gave 99.9% control of the mealybugs, *Pseudococcus njalensis* (Laing) and *P. citri* (Risso), both vectors of the swollen-shoot virus of cacao in West Africa. Dimefox was found to protect against the mealybugs for as long as 6 weeks after a single treatment and for several months with repeated applications. When this chemical was applied by trunk implantation (at 250 p.p.m. of the estimated aerial weight of the tree), the most effective control of mealybugs was obtained on branches directly above the sites of implantation, indicating that little radial distribution took place. Determination of dimefox residues in the cacao beans by the method of Dupee *et al.* (1956) demonstrated the presence of 0.01 to 0.03 p.p.m. of dimefox 21 days after treatment (Ripper 1957). Hanna & Heatherington (1957) demonstrated that repeated applications of dimefox as a soil treatment in combination with the practice of cutting out diseased cacao trees gave a greater reduction in the incidence of the swollen-shoot virus disease than the cutting out method alone.

In this investigation, the rate of translocation, persistence in the foliage, and residues or off-flavors in cacao beans following implantation of systemic insecticides other than dimefox were studied. The field studies were conducted at the Inter-American Institute of Agricultural Sciences at Turrialba, Costa Rica and the chemical synthesis and analytical work were conducted primarily at the University of Wisconsin, Madison, Wisconsin.

Translocation and Persistence of Systemic Insecticides in Cacao Foliage.—*Preliminary screening studies with seedling cacao.*—Young seedling cacao were used for a preliminary study of the relative rate of absorption of 14 systemic insecticides through the roots. The compounds tested were Thimet (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate), Am. Cyanamid 12009 (*O,O*-diethyl *S*-(propylthio)methyl phosphorodithioate), Bayer 19639 (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothioate), Bayer 23129 (*O,O*-dimethyl *S*-2-(ethylthio)ethyl phosphorodithioate), demeton (mixture of *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothiolate and *O,O*-diethyl *O*-2-(ethylthio)ethyl phosphorothiolate), methyl demeton (mixture of *O,O*-dimethyl *S*-2-(ethylthio)ethyl phosphorothiolate and *O,O*-dimethyl *O*-2-(ethylthio)ethyl phosphorothiolate), Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate as the hydrogen oxalate salt), schradan (octamethyl pyrophosphoramide), dimefox, Phosdrin (*O,O*-diemethyl 1-methoxycarbonyl-1-propen-2-yl phosphate), Shell OS 1836 (*O,O*-diethyl 2-chlorovinyl phosphate), Shell OS 1808 (*O,O*,-diethyl 1-ethoxycarbonyl-1-propen-2-yl phosphate), and Isolan (dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate). Young potted cacao seedlings 18 to 24 inches tall were treated by soil drench with 10 mg. of each compound as either a water solution or dilute emulsion for each seedling. Analyses of the foliage for anticholinesterase agents (method of Casida *et al.* 1956) over a 12-day period indicated the highest amounts of inhibitors with Thimet, Am. Cyanamid 12008 and 12009, Chipman R-6199, demeton and Isolan. Young coffee seedlings were treated with the same compounds and assayed in a similar manner. The

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by the Gretchen B. Schoenleber Cocoa Research Grant and by a grant from the U. S. Atomic Energy Commission (Contract No. AT(11-1), Project No. 14). Accepted for publication May 12, 1958.

[2] Present address: Hazleton Laboratories Inc., Falls Church, Virginia.

[3] With the cooperation of the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica.

The authors are indebted to Mr. L. Schwarz of the American Cocoa Research Institute, Washington, D. C., to Drs. L. R. Cook and R. F. Korfhage of the Ambrosia Chocolate Company, Milwaukee, Wisconsin, to Dr. N. W. Kempf of the Walter Baker Division, General Foods Corporation, Dorchester, Massachusetts, and to Drs. T. C. Allen and R. D. Shenefelt of the University of Wisconsin, Madison for many helpful discussions and assistance in the organization and accomplishment of this investigation. The excellent technical aid of Mrs. J. S. Bowman, Mrs. J. E. Casida, Mr. and Mrs. D. Pokorski and Miss Anda Dimze is also acknowledged.

Without the enthusiastic and whole-hearted cooperation of Dr. R. Allee, Dr. H. Kroening, Dr. F. Weliman, Mr. A. Parades, Mr. M. Machicado and particularly Drs. J. Orsenigo, J. Leon and G. Havord this study would not have been possible. Our indebtedness for their generous cooperation is gratefully acknowledged.

Table 1.—Upward translocation and radial distribution of Thimet-P^{32} in young cacao plants treated by trunk implantation using from one to four implantation holes at the rate of 25 mg. actual Thimet-P^{32} per hole.

Inches from Site of Implantation	Radial Position from Holes	Relative P^{32} in Leaves at Indicated Days				
		3	5	14	20	27
1–5	above[a]	9	21	52	74	100
	adjacent[b]	—	—	—	—	—
	opposite[c]	1.5	—	13	16	—
5–10	above	9	24	50	64	67
	adjacent	2.2	—	17	22	23
	opposite	1.3	—	6	6	—
10–15	above	6	20	41	48	64
	adjacent	2.0	—	16	16	—
	opposite	1.0	—	2.5	5	—
15–20	above	5	20	40	50	59
	adjacent	1.5	—	13	15	18
	opposite	0.7	—	6	8	—
20–25	above	3	13	43	54	52
	adjacent	1.0	—	15	18	20
	opposite	—	—	—	—	—
25–30[d]	above	—	7	37	54	52
	adjacent	—	—	—	—	—
	opposite	—	—	—	—	—

[a] Based on plants with 1, 2, 3, and 4 implantation holes.
[b] Based on proportionate radioactivity in the "adjacent" compared with the "above" leaves in plants with 1 and 2 implantation holes.
[c] Based on proportionate radioactivity in the "opposite" compared with the "above" leaves in the plant with one implantation hole.
[d] Includes all leaves to tip except flush leaves (see text).

order of activity of the compounds was essentially the same after absorption by coffee but in all cases less anticholinesterase agents were present in the coffee than in the cacao. This was probably due to less efficient absorption because when coffee and cacao seedlings were placed with the roots in a solution of radioactive orthophosphoric acid the rate of uptake was much greater with the cacao seedlings. On the basis of these preliminary studies, Thimet, Chipman R-6199, demeton, and Isolan were selected for study in mature cacao trees along with the previously tested (Hanna *et al.* 1955) dimefox.

Upward translocation of Thimet-P^{32} in cacao plants.—The rate of upward translocation and radial distribution of Thimet-P^{32} (synthesized according to the method of Bowman & Casida, 1957) were studied in cacao plants under greenhouse conditions in Madison, Wisconsin. Four young cacao trees about 30 inches tall were treated by implanting Thimet-P^{32} in the base of their trunks using from one to four implantation holes and 25 mg. of insecticide per hole. The holes were radially located at 90° from each other in the trees treated with two, three, and four holes. Radioassay of the leaves was accomplished by periodically counting the individual intact leaves on the four trees with a shielded Geiger-Muller tube. The leaves were designated as "above," "adjacent," or "opposite" according to their radial position by quadrants with respect to the implantation holes (table 1).

In general, upward translocation of the phosphorus-32 from Thimet was slow, with the leaves in the lower 20 inches of the plants increasing in concentration of radioactive materials by about 10-fold from the third to the 27th day after treatment. When the total of the data are considered for the entire experimental period, leaves located adjacent to the upward translocation flow possessed 32% of the radioactivity found in leaves directly above the implantation holes, while leaves located opposite the holes possessed only 15% of the material above the implantation sites. Throughout the experiment, the highest concentration of insecticide was located in leaves on the lower 10 inches of the plants and 20 days after treatment the radioactivity in the upper 20 to 30 inches of the plants appeared to have equilibrated since the concentration was almost the same at 20 and 27 days after treatment. The apical flush leaves remained relatively low in radioactivity while in their red succulent condition but increased in radioactivity when they hardened and became green.

Translocation and distribution of Chipman R-6200-P^{32} in the foliage of mature cacao trees.—Radioactive Chipman R-6200 (*O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate as the *p*-toluene sulfonate salt) was prepared by reacting diethyl phosphoryl-P^{32} chloride (Forbes *et al.* 1946, McCombie *et al.* 1945) with sodium beta-diethylaminoethylthiolate in benzene solution (Ghosh 1955). After chromatographic purification (Bowman & Casida 1957), the *p*-toluenesulfonate salt was formed in and crystallized from acetone. Three cacao trees (3-inch trunk diameters) were treated by trunk implantation (Ripper 1957) with one gram of the insecticide per hole and three holes per tree. Two trees treated were from clone 650 (Trees A and C) and one from clone 11 (Tree B) (United Fruit Company, Costa Rica) and all were heavily set and beginning to bear ripe pods. At weekly intervals, leaf samples selected at random from each of the branches of the trees were analyzed for total radioactivity. Thirty grams of leaf material were macerated in a Waring blender with 100 ml. of water and the solid residue separated from the liquid phase by straining through cheesecloth. Where sufficient radioactivity was obtained in the liquid phase, partitions between chloroform and water at pH 8 were conducted to determine the nature of the radioactive material. Under these conditions, the free base of Chipman R-6200 partitioned 100% into chloroform and the hydrolysis products remained in the water phase.

The results of these analyses are shown in table 2 and figure 1. Over the 8-week period following trunk implantation there was a steady increase in the total equivalents of Chipman R-6200 in the foliage of cacao trees and a definite increase in the chloroform/water partition ratio for the radioactivity. One explanation for this proportionate increase in chloroform-soluble material might be that the plant utilized the water-soluble or hydrolyzed materials more rapidly in the formation of phospholipids and phosphoproteins than the chloroform-soluble insecticide was hydrolyzed. Thus, the hydrolysis products would become bound as an unextractable form, leaving a higher percentage of the soluble-residues as the chloroform-extractable R-6200 base.

A great variation occurred in the distribution of the radioactivity from R-6200-P^{32} throughout the cacao trees (fig. 1). No definite distribution pattern is evident with

the possible exception in Tree C where a relatively low amount of translocation took place into branches that had had their tips pruned. Branches varied in the radioactive content of their leaves (weekly averages over an 8-week period) by a factor of 6.3 for Tree A, 4.4 for Tree B, and 21 for Tree C. Apparently there was very little radial translocation from this method of treatment and a method yielding better coverage is needed.

An attempt was made to characterize the radioactive chloroform-soluble material in the cacao foliage 8 weeks after implantation and 9.7 mg. of Chipman R-6200 equivalents were recovered (by chloroform extraction as previously described) from 889 grams of leaf material. Clean-up of the radioactive material was accomplished by partitioning the chloroform extract with 1% hydrochloric acid to form the water-soluble hydrochloride salt, then adjusting the aqueous phase to pH 8 and re-extracting with chloroform to recover most of the radioactivity. The radioactive materials were then chromatographed on a celite-isooctane-methanol column (Bowman & Casida 1957) and the major peak again purified by extraction into acid from chloroform and then back into chloroform from pH 8. An infrared spectrum obtained on the product and its partitioning properties on purification indicated that the radioactive material was *O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate. The chloroform-soluble radioactivity 2 months after treatment, therefore, represented the free base of Chipman R-6200.

Persistence of other systemic insecticides in the leaves of mature cacao trees.—The rate of uptake and persistence of anticholinesterase agents in the foliage of mature cacao trees in Costa Rica treated with non-radioactive Thimet, dimefox, demeton, Isolan, and Chipman R-6199 were determined. Implantation of the insecticides consisted of boring four holes equidistant around the base of the trunks about 1 foot above the ground level. The holes were one-fourth inch in diameter, from 2 to 4 inches deep, and at a 45° angle to the main axis of the tree. Two grams of actual toxicant were pipetted into each hole except with R-6199 which was applied as a 10% water solution at the rate of 0.2 grams per hole. The holes were then plugged with corks.

Leaf homogenates were assayed for anticholinesterase agents by a described procedure (Casida *et al.* 1956). All leaves were thoroughly washed with water before assaying to remove Bordeaux mixture from their surfaces.

Table 2.—Translocation and persistence of Chipman R-6200-P^{32} in the foliage of three cacao trees following trunk implantation of 3 grams per tree.

Weeks After Treatment	Fraction	Average p.p.m. Chipman R-6200 Equivalents		
		Tree A	Tree B	Tree C
1	Chloroform	2.8	1.1	—
	Water	5.5	9.6	—
	Residue[a]	16	19	—
	Range[b]	(11 – 47)	(7.5– 51)	—
2	Chloroform	6.1	1.9	8.8
	Water	8.4	12	2.5
	Residue	18	21	18
	Range	(11 – 75)	(10 – 87)	(2.0– 90)
3	Chloroform	6.3	2.1	9.6
	Water	6.3	9.5	4.4
	Residue	22	24	22
	Range	(6.0– 76)	(10 – 82)	(2.9– 91)
4	Chloroform	12	2.4	8.6
	Water	8.5	15	2.9
	Residue	31	24	25
	Range	(12 –133)	(11 – 96)	(5.7– 89)
5	Chloroform	12	8.1	13
	Water	8.3	17	5.4
	Residue	37	37	31
	Range	(12 –149)	(21 –118)	(8.2–101)
6	Chloroform	18	5.9	11
	Water	11	7.7	6.3
	Residue	55	28	36
	Range	(19 –158)	(18 – 85)	(11 – 97)
7	Chloroform	17	8.1	—
	Water	11	11	—
	Residue	55	32	—
	Range	(21 –159)	(21 –118)	—
8	Chloroform	33	7.8	—
	Water	8.6	8.8	—
	Residue	62	28	—
	Range	(53 –210)	(14 –118)	—

[a] Unextractable radioactive material remaining in pulp.
[b] Variation among branches in the total p.p.m. of Chipman R-6200 equivalents in the foliage.

Analyses were conducted on each of 16 trees treated with Thimet, dimefox, demeton, and Chipman R6199 and

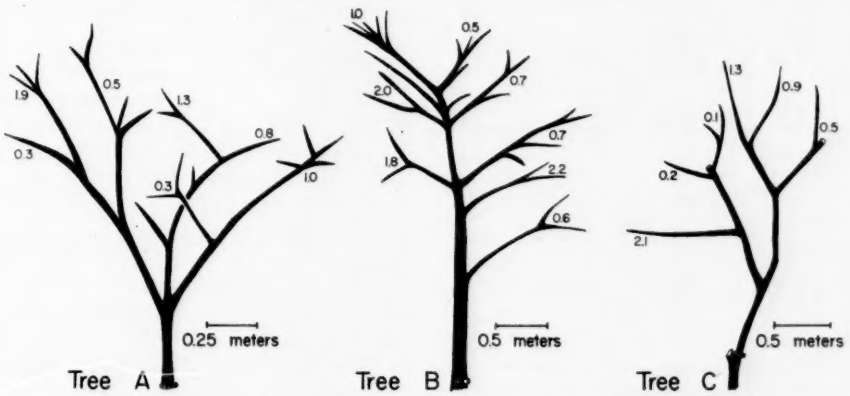


FIG. 1. Relative distribution of Chipman R-6200-P^{32} among the branches of three cacao trees over an 8-week period after trunk implantation with 3 grams of the toxicant per tree in three holes.

Table 3.—Translocation and persistence of systemic insecticides in the foliage of cacao trees treated by the implantation method.

Treatment	Grams per Tree	Per Cent Cholinesterase Inhibition[a] After		
		3 Weeks	20 Weeks	63 Weeks
Thimet	8.0	34	40	47
Dimefox	8.0	38	3	1
Demeton	8.0	53	22	5
Isolan	8.0	46	17	5
Chipman R-6199	0.8	23	48	47

[a] Aliquot of homogenate representing 1.17 grams leaf tissue adjusted to pH 8.0, added to 1.5 ml. whole human blood at pH 8.0, made up to 5 ml. total volume, incubated for 1 hour at 37° C. for inhibitor to combine with enzyme then assayed for residual cholinesterase activity.

eight trees treated with Isolan at 3, 20, and 63 weeks after treatment.

Anticholinesterase agents were present in the leaves of all treatments 3 weeks after implantation (table 3). Inhibitors in Thimet and Chipman R-6199-treated trees were still detectable 63 weeks after treatment whereas demeton- and Isolan-treated trees steadily decreased in anticholinesterase agents after the initial uptake period. Foliage from dimefox trees 20 weeks after treatment did not significantly inhibit cholinesterase but dimefox is very low in anticholinesterase activity and its active metabolites in plants are very unstable (Arthur & Casida 1958).

Similar analyses were conducted on other trees 86 weeks after treatment with 0.8 grams of insecticide per tree. Three replicates of Thimet and Chipman R-6199 gave an average of 35 and 11% inhibition, respectively, from 1.17 grams of plant tissue. The long persistence of anticholinesterase agents in the foliar parts of cacao trees after a single trunk implantation treatment with Thimet and R-6199 warrants further investigation into the insecticidal properties of these compounds for controlling the foliage feeding pests of cacao.

RESIDUES IN THE CACAO PODS AND BEANS.—*Studies with Chipman R-6200-P*^{32}.—Pods from the trees treated with radioactive Chipman R-6200 were identified as to maturity, location on the major branches, and distance from the implantation sites. These pods were harvested between the sixth and eighth weeks after treatment, separated into the various tissue components, and analyzed (table 4). With the exception of the hull, the tissues were macerated with water in a Waring blender, strained through cheesecloth, and radioassays were made on aliquots of both the liquid and solid residue. When the liquid extract contained sufficient radioactivity, partitions between chloroform and water at pH 8.0 were performed to determine the nature of the radioactive residues. The hull tissue was analyzed by radioassays on 1-gram samples of the whole tissue. The total P^{32} residues were highest in the cotyledons where up to 114 p.p.m. of R-6200-equivalents were detected. However, partitioning properties of these residues indicated that they were all hydrolyzed products of R-6200 and could, therefore, be considered as presenting no toxic hazard. Relatively low residues were found in the seed coats and placental tissue, whereas, the hull contained as high as 23 p.p.m. of radioactive residues. The nature of the residues in the hull is not known but might be of importance in controlling insects which surface-feed on the pods. No correlation was apparent between the location of the pods on the trees with respect to the implantation sites and the amount of residue they contained.

The radioactive distribution in the stems upon which the pods were borne was determined. The cortical tissues of the stems were stripped from the xylem and assays of the total radioactivity determined on both tissues (table 4). On the average, 46% of the radioactive materials in the stems were located in the xylem tissue and the remaining 54% in the cortical tissues. The nature of these residues is not known but should they be toxic to insects they might be of importance in controlling certain bark-

Table 4.—Chipman R-6200 and hydrolysis products in various parts of cacao pods 6 to 8 weeks after treatment by trunk implantation with 3 grams R-6200-P^{32} per tree.

Stage of Pod Development	Tree	No. of Pods	Average Per Cent of the P^{32} in the Pod Stems Appearing in Xylem	Average and Range of Total P.p.m. R-6200 Equivalents			
				Cotyledons	Mucilage and Seed Coat	Placenta	Hull
Mature	A	1	38	41[a]	1.4	2.7	7.4
	B	8	—	6.6[a] (0.2–30)	1.4 (0.3–3.5)	1.1 (0.1–2.4)	2.0 (0.4–7.9)
Intermediate	A	5	49	42[a] (26–75)	5.8[a] (1.8–12)		6.4 (3.0–9.8)
	B	2	46	72[a] (30–114)	2.1 (0.0–4.2)		8.7 (3.6–14)
	C	4	49	20[a] (5.6–35)	0.5 (0.2–1.0)		1.0 (0.5–1.7)
Young	A	16	53		11 (1.2–31)		6.0 (1.3–23)
	B	5	43		8.0 (5.1–10)		7.9 (5.4–11)
	C	8	43		10 (4.1–26)		3.5 (0.3–10)

[a] Partitions between chloroform and the water extract at pH 8.0 gave 100% recovery in the aqueous phase.

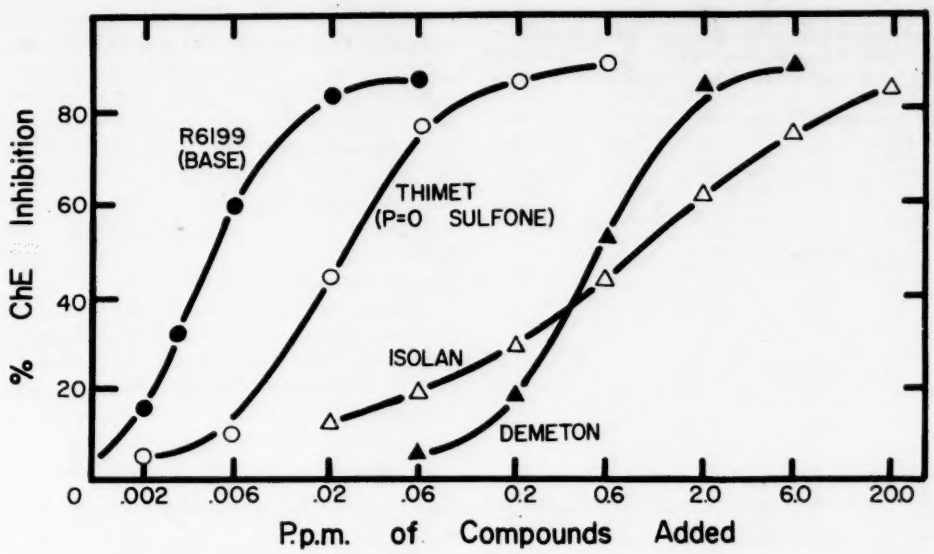


FIG. 2. Recovery of anticholinesterase agents of Thimet (oxygen analog sulfone), Chipman R-6199 (free base), demeton, and Isolan when added in known amounts to untreated cacao beans and extracted by the described method.

feeding insects.

Extraction of cacao beans from treated-trees and analysis for anticholinesterase agents.—The pods from trees treated in Costa Rica were harvested and the mature cacao beans were sent by air mail to Wisconsin. The beans from single pod samples were washed, dried at 50° C. for 12 hours, and roasted in a laboratory roaster (Jabez Burns and Sons) at about 230° F. for 20 minutes. Each sample was then ground in a mortar, mixed with an equal weight of anhydrous sodium sulfate, and extracted for 24 hours with 200 ml. of Skelly-B in a soxhlet extractor. The resulting Skelly-B extract was partitioned twice with an equal volume of acetonitrile, leaving most of the cocoa butter in the Skelly-B phase and recovering almost all the anticholinesterase agents in the acetonitrile. Further clean-up consisted of evaporating the acetonitrile, washing the residue three times with 5 ml. portions of 90% methyl alcohol, filtering these washes, and evaporating off the alcohol. The resulting residue was dissolved in 5 ml. of acetone and aliquots of up to 2.0 ml. were transferred to assay tubes, the acetone evaporated, and the residue analyzed for the presence of anticholinesterase agents by the method described previously for the foliage. Proofing of this extraction system with radioactive Thimet added at 1 to 10 p.p.m. to untreated cacao beans showed 68% recovery of the initial radioactivity.

Recovery analyses by the anticholinesterase method were made on Chipman R-6199 as the free base and as the oxalate salt, the oxygen analog sulfone of Thimet (*O,O*-diethyl *S*-(ethylsulfonyl)methyl phosphorothiolate), demeton, and Isolan by adding known amounts of each compound directly to untreated cacao beans in the soxhlet extractors. The recovery samples were then run in duplicate by the described extraction, clean-up, and assay procedure (fig. 2). Identical inhibition results were obtained with equimolar amounts of Chipman R-6199 as the free base and as the oxalate salt.

The results of the residue analyses of beans from treated cacao trees are presented in table 5. A very high degree of sensitivity was obtained with Chipman R-6199 free base and Thimet oxygen analog sulfone (fig. 2) whereas dimefox was not detectable at 100 p.p.m. In most of the pods from all the treatments the residues were below the sensitivity limit of the method. Where residues were found the highest values from individual pods from Chipman R-6199-, Thimet-, demeton-, and Isolan-treated trees were 0.008, 0.026, 0.22, and 0.21 p.p.m., respectively.

A record of the location of each pod on the trees was kept and the distance from the site of implantation was not found to be a major factor in the distribution of the insecticides into the pods. Pods located on the trunks a few centimeters from the site of implantation were as frequently without residues as those located out on the branches. Additional support was given to these findings when individual branches of cacao trees with pods in all stages of development were treated with each of the above insecticides at 4 grams of actual compound per branch and no residues were detected in any of the 52 pods analyzed from all treatments regardless of their distance from the implantation site.

Table 5.—Residue analyses of single pod samples of ripe cacao beans selected at random 2 to 6 months after treatment from trees (8–16 per treatment) treated by implantation of 8 grams of actual insecticide.[a]

Treatment	AntiChE Agent[b]	Pods with Residues		Pods without Residues	
		No.	Range (p.p.m.)	No.	Sensitivity Limit (p.p.m.)
Chipman R-6199[c]	free base	9	0.001–0.008	44	0.001
Thimet	P=Osulfone	11	0.01 –0.026	42	0.01
Demeton	P=O sulfide in technical mixture	11	0.1 –0.22	50	0.1
Isolan	*per se*	2	0.1 –0.21	26	0.1

[a] Dimefox produced no ChE inhibition at 100 p.p.m.; analysis of 52 pods from dimefox-treated trees showed no antiChE agents present.
[b] Used in preparing standard curve (fig. 2).
[c] Treated with 0.8 gram actual insecticide per tree.

Table 6.—Flavor evaluations, anticholinesterase agents present in cacao beans and chocolate bars processed from these beans, and anticholinesterase agents in the foliage of trees treated by trunk implantation in Costa Rica at 8 grams actual toxicant per tree.

Treatment	Per Cent ChE Inhibition by 1.17 Gm. Leaf Tissue	P.p.m. AntiChE Agents		Flavor Evaluations	
		Beans	Bars	Mean[a]	Duncan's Test (5% signif. level)
1.5 months after treatment					
Chipman R-6200	99	0.004	<0.001	6.6	.
Dimefox	1	<100	<100	6.6	.
Thimet	48	<0.01	0.014	6.4	. .
Demeton	71	<0.10	<0.10	6.3	. .
Control	—	—	—	6.1	. .
BHC	—	—	—	5.1[b]	.
3 months after treatment					
Chipman R-6200	90	<0.001	<0.001	7.1	.
Thimet	42	<0.01	<0.01	7.0	.
Dimefox	9	<100	<100	6.6	. .
Demeton	49	<0.10	<0.10	6.3	. .
Control	—	—	—	5.4	. .
BHC	—	—	—	3.4[b]	.

[a] The average of the mean ranks supplied by the following industrial taste panels: Walter Baker Division, General Foods Corp., Hershey Cocolate Co., Ambrosia Chocolate Co., Nestlés Chocolate Co., Klein Chocolate Co. and Blumenthal Chocolate Co.

[b] Described as musty, sharp, and earthy.

Flavor evaluations.—Because of the high concentration of insecticide hydrolysis products found in the cotyledons of the cacao beans in the Chipman R-6200-P^{32} studies, an experiment was designed to determine if any off-flavors might result from their presence. In September, 1957 implantation applications of Thimet, dimefox, demeton, and Chipman R-6200 were made to mature cacao trees in Costa Rica at the rate of 8 grams actual material per tree with five single tree replicates for each treatment. Five trees were also sprayed to run-off with BHC (Isotox Spray No. 200, 20% gamma isomer from lindane, 80% inert materials) at the rate of 2 lbs. gamma isomer per 100 gal. water, a concentration which is several times greater than that used in areas such as West Africa. An additional five trees were left untreated to serve as controls. Six mature cacao pods per tree (30 pods per treatment) were collected at 1.5 and 3 months after treatment. The cacao beans from the trees within each treatment were pooled, placed in onion sacks, and fermented for 6 days in fermentation boxes along with untreated cacao beans from the same production area. The fermented beans were dried and sent by air mail to the University of Wisconsin where subsamples were removed for residue analyses and the remainder of the beans were sent to the Walter Baker Division, General Foods Corporation, where they were processed into chocolate bars for flavor evaluation.

Anticholinesterase analyses were conducted on the roasted cacao beans and the processed chocolate bars using the Skelly-B extraction technique previously described and on leaf samples taken at the same time from the trees. The flavor evaluations were conducted by a series of taste panels organized by the American Cocao Research Institute (see table 6). The average flavor rank given the treatments by each combined panel was used to evaluate the significance of off-flavoring.

High concentrations of anticholinesterase agents were present in the leaves in all the organophosphate treatments except dimefox at both 1.5 and 3 months after treatment (table 6). In most cases, no anticholinesterase agents were detected in the cacao beans or in the processed chocolate bars. Where anticholinesterase agents were detected, they were of a very low magnitude.

The flavor evaluations were subjected to Duncan's multiple range test (Duncan 1955) to determine differences between the mean rank for each treatment at the 5% level of significance. In both the 1.5- and 3-month samples, BHC was significantly different from the remaining treatments and was described as possessing a definite "musty," "sharp," or "earthy" flavor. All systemic treatments were ranked higher than the control though not significantly different.

Hydrolysis of Chipman R-6200 during fermentation.—Most commercial cacao beans are subjected to fermentation for periods of from 3 to 7 days at temperatures up to 50° C. (Wadsworth & Howat 1954). The stability of Chipman R-6200 in the mucilaginous matrix of ripe cacao beans was determined under simulated fermentation conditions. To each of three quart jars filled with fresh cacao beans from ripe pods was added 142 mg. of Chipman R-6200-P^{32} in 20 ml. water. The beans were incubated at a constant 41° C. and after various time intervals the beans were stirred (to prevent clumping and to insure a uniform mixture) and a 3.0-ml. aliquot of the run-off liquid was taken from the bottom of the jars. The pH was determined and each sample was then adjusted to pH 8.0 and divided into three sub-samples which were partitioned with equal volumes of cholorform to determine the per cent hydrolysis (the radioactivity appearing in the aqueous layer) (fig. 3).

The pH of the run-off liquid was observed to increase during the 8-day fermentation period. Little if any change occurred in the Chipman R-6200 hydrolysis rate during the 8 days despite this slight rise in pH. R-6200 was only slowly hydrolyzed under these conditions of fermentation and little would be lost from this source within the time used in commercial fermentation.

Discussion.—The systemic insecticides Thimet and Chipman R-6200, when implanted into the trunks of cacao trees, are readily translocated into the foliar parts of the trees and persist for periods up to 20 months after treatment. Little or no insecticide residues were found in the cacao beans at any time after treatment regardless of the pod position on the trees in relation to the site of implantation of the insecticides. Radioactive studies with R-6200-P^{32} demonstrated a high concentration of phosphorus-containing compounds in the cotyledons of the cacao beans but these materials did not partition into chloroform and did not inhibit cholinesterase and therefore cannot be considered as being hazardous residues. No off-flavors were found in chocolate made from beans harvested from any of the systemic-treated trees. Since Chipman R-6200 and Thimet result in neither toxic residues nor off-flavors it should be of interest to test these systemics in cacao production areas that are threatened by the attack of mealybugs, capsid bugs, or other pests. However, the possible effect of the implantation holes in serving as a site for the entry of pathogenic organisms or in otherwise affecting the health of the tree has not been determined.

No deleterious phytotoxic effects were displayed by either Thimet or R-6200 when applied as trunk implantations or soil drenches. On the contrary, there was some evidence that Chipman R-6199 and R-6200 stimulated

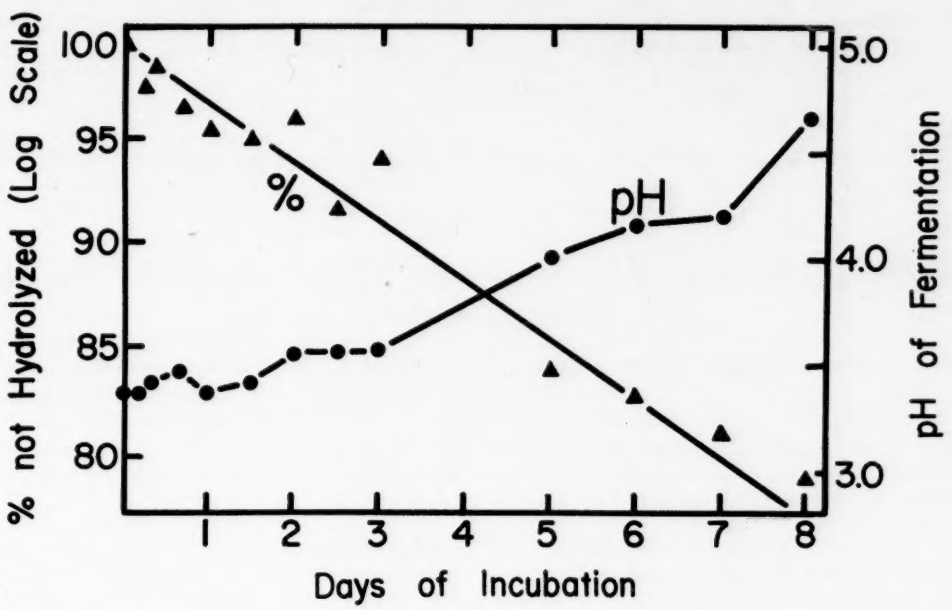


FIG. 3. Hydrolysis rate of Chipman R-6200 under cacao fermentation conditions.

plant growth. However, it was observed that when one main leader of a tree was treated by implantation of R-6200, there was a delay in the maturity of the pods on the treated portion of the plant as compared with the untreated portion.

Thimet, when used as a seed treatment by coating cacao beans at the rate of 10% by weight with 50% Thimet on charcoal, caused a definite delay in germination, a great reduction in survival, and the resulting plants appeared abnormal due to definite stunting of the internodes. This deleterious effect on germination and growth was not observed when beans were treated with 10% Thimet on charcoal at the rate of 10% of the weight of the fresh beans. The level of anticholinesterase agents in the leaves of these plants was insignificant 9 months after treatment.

Insect populations in the experimental area in Costa Rica were inadequate to obtain comparative insect control data on the compounds. Several attempts were made to obtain preliminary control data using young cacao plants infested with *Pseudococcus citri* (Risso) under greenhouse conditions at the University of Wisconsin. Infested plants were treated with dimefox, Chipman R-6200, and Thimet by soil drench at the rate of 500 mg. of actual toxicant per tree and by trunk implantation at the rate of 100 mg. per tree in four implantation holes. Two days after treatment the mealybugs on the dimefox-treated plants had completely evacuated the plants. Extreme phytotoxicity resulted from this dimefox treatment with complete defoliation occurring within 1 week after treatment. The condition of the plants may have contributed to the migration of the mealybugs. Thimet-treated plants, on the other hand, displayed complete control of the mealybugs 2 weeks after treatment and no phytotoxicity was observed. Plants treated with Chipman R-6200 displayed a stimulation of growth and no apparent decrease in the mealybug population. However, the final test for any comparative insect control experiment must be conducted under field conditions in areas where high populations of destructive insects are present.

REFERENCES CITED

Arthur, B. W., and J. E. Casida. 1958. Biological and chemical oxidation of tetramethyl phosphorodiamidic fluoride (Dimefox). Jour. Econ. Ent. **51**: 49–56.

Bowman, J. S., and J. E. Casida. 1957. Metabolism of the systemic insecticide *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate (Thimet) in plants. Jour. Agric. Food Chem. **5**: 192–7.

Casida, J. E., P. E. Gatterdam, L. W. Getzin, Jr., and R. K. Chapman. 1956. Residual properties of the systemic insecticide *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate. Jour. Agric. Food Chem. **4**: 236–43.

Duncan, D. B. 1955. Multiple range and multiple F test. Biometrics **11**: 1–42.

Dupee, L. F., D. F. Heath, and I. K. H. Otter. 1956. Determining traces of tetramethyl phosphorodiamidic fluoride (Dimefox) in crops. Jour. Agric. Food Chem. **4**: 233–6.

Forbes, M. C., C. A. Roswell, and R. N. Maxson. 1946. Phosphorus (III) trichloride. Inorganic Synthesis **2**: 145–7.

Ghosh, R. Personal communication. November, 1955.

Hanna, A. D. 1954a. Research in the use of systemic insecticides to control the mealybug vectors of the swollen-shoot disease in cacao. Phytiat.-Phytopharm. Spec. Issue. 426–32.

Hanna, A. D., and W. Heatherington. 1957. Arrest of the swollen-shoot virus of cacao in the Gold Coast by controlling the mealybug vectors with the systemic insecticide, dimefox. Ann. Appl. Biol. **45**: 473–80.

Hanna, A. D., W. Heatherington, and E. Judenko. 1952. Control of the mealybug vectors of the swollen-shoot virus by a systemic insecticide. Nature **169**: 334–5.

Hanna, A. D., E. Judenko, and W. Heatherington. 1955. Systemic insecticides for the control of insects trans-

mitting swollen-shoot virus diseases of cacao in the Gold Coast. Bull. Ent. Res. 46: 669–710.

Hanna, A. D., and J. Nicol. 1954b. Application of a systemic insecticide by trunk implantation to control a mealybug vector of the cacao swollen-shoot virus. Nature 173: 730–2.

McCombie, I. H., B. C. Saunders, and G. J. Stacey. 1945. Esters containing phosphorus. Part I. Jour. Chem. Soc. 1945: 380–2.

Ripper, W. E. 1957. The status of systemic insecticides in pest control practices. Adv. Pest Control Res., Vol. 1, Interscience Publ. Co., New York.

Wadsworth, R. V., and G. R. Howat. 1954. Cocoa fermentation. Nature 174: 392–4.

Studies on the Use of the Brine Shrimp *Artemia salina* (Leach) as a Test Organism for Bioassay[1]

WALLACE A. TARPLEY,[2] *Department of Entomology and Zoology, Clemson College, Clemson, S.C.*

ABSTRACT

Attempts were made to utilize a fresh water form of the order Cladocera, two common forms of hydra, *Pelmatohydra oligactis* and *Hydra viridissima*, and the brine shrimp, *Artemia salina* (Leach), to determine insecticide residues. The brine shrimp was the only one that proved to be suitable for use in bioassay work.

Tests were conducted to determine the optimum type and amount of food, optimum type and concentration of salt solution in which the specimens were reared, and the temperature that would consistently give brine shrimp of equivalent size and susceptibility to insecticide concentrations at various stages of development. For cultures of approximately 500 individuals, 150 mgs. per day of "Food for Brine Shrimp" appeared to be the optimum amount. Of three forms of salt, aquarium salt, table salt, and rock salt, the latter consistently proved to be the best, and was used in rearing all cultures employed in bioassay work. The optimum salt concentration was 3.5%. The optimum temperature was 27.5° C.

Mortality of the brine shrimp was determined after 5 hours exposure to the test solution with the aid of a counting device designed for that purpose. Insecticides used in the tests were DDT, parathion and dieldrin. All compounds were run simultaneously on each of 6 days against a given instar, using approximately 30 brine shrimp for each insecticide concentration. Check mortality was negligible. The susceptibility of the brine shrimp to DDT, parathion, and dieldrin was: DDT>parathion>dieldrin. The results indicate brine shrimp was less sensitive to insecticides than larvae of *Aedes aegypti* (L.).

In an effort to overcome the difficulty and minimize the time involved in maintaining a constant source of test specimens of those species normally used for bioassay purposes, research was conducted on the brine shrimp, *Artemia salina* (Leach) to determine its feasibility as a substitute bioassay test specimen. Earlier efforts indicated that two common species of hydra, *Pelmathohydra oligactis* and *Hydra viridissima*, and one species of Cladocera were unsuitable for bioassay work. It was virtually impossible to maintain a continuous culture of Cladocera under laboratory conditions. Peculiar habits of the hydra, such as contracting in response to various stimuli into a small mass scarcely larger than the head of a pin and attaching themselves so firmly to objects that it is extremely difficult to transfer them from one container to another, make them impractical for bioassay work even if they respond adequately to various insecticides. Determination of the feasibility of utilizing brine shrimp in the detection of insecticide residues necessarily entailed the discovery of optimum environmental conditions, types and concentrations of substances to which various instars of brine shrimp are susceptible, and the development of testing techniques and equipment.

REVIEW OF LITERATURE.—The brine shrimp has been observed and studied for some 70 years, particularly by physiologists. This is probably due to its characteristic features which make it valuable in the study of physiological and genetic relationships. Its relative transparency and the morphological variations exhibited in varying salt concentrations make it particularly appealing to physiologists. It has recently endeared itself to geneticists by its variable chromosome number.

Until recently the genus *Artemia* was believed to be represented in the continental United States by four species. The present consensus, according to Pennak (1953), is that there is a single cosmopolitan species, *Artemia salina*, which has numerous intergrading physiological and morphological varieties.

Coincident with the inception of the possible use of *Artemia salina* in bioassay work in December 1954, the author initiated an extensive search of available literature to ascertain if any work had ever been conducted on this species, especially in relation to biological assays. At that time no references to the use of brine shrimp in detecting insecticide residues were found. A short note found in Farm Chemicals (Anonymous 1955) first announced the possibility of the brine shrimp as an organism for testing the presence of insecticide residues. A later and more detailed report of this same work was given by Michael *et al.* (1956). They reported *Artemia salina* as being sensitive to chlordane, methoxychlor, lindane, toxaphene, and DDT at concentrations of 0.01 parts per million. In measuring toxicity, various age groups of brine shrimp were tested by being placed directly in the suspension of the test chemical. The swimming movements of an affected individual were rapidly curtailed, resulting in its sinking

[1] Technical contribution No. 284 of the South Carolina Agricultural Experiment Station, Clemson. Portion of a thesis presented for the degree of Master of Science at the Clemson Agricultural College. Accepted for publication May 15, 1958.

[2] Present address: Zoology Department, University of Georgia, Athens, Georgia. The author wishes to express his most sincere appreciation to Professor R. E. Ware, and to Drs. J. H. Cochran and J. K. Reed of the Entomology and Zoology Department, Clemson College, for their valuable criticisms and suggestions during the progress of this work.

to the bottom of the container. Using this response as the criterion, readings were usually obtained in 45 minutes to 2 hours. Adult brine shrimp reacted to all of the above-mentioned insecticides at concentrations of 0.01 parts per million within 45 to 180 minutes, with the exception of toxaphene which required 18 hours. The authors reported initial tests using phototaxy as a physiological criterion for mortality determination as being promising.

LIFE HISTORY.—When reared in 3.5% saline solution the males are 6 to 8 mm. in length and the females 8 to 10 mm. At this saline concentration the brine shrimp reach sexual maturity, as evidenced by copulation, in about 21 days and normally live for 4 or more months. The two sexes occur in about equal numbers. According to Lochhead (1941), they reproduce parthenogenetically as well as sexually, occasionally producing excysted eggs. These have the power of lying dormant for as long as 15 years, as well as withstanding extremes in temperature, pressure, and salt concentrations. These qualities are highly desirable in a species useful in biological assays.

The total number of molts is never constant, even under natural conditions, though 12 is the normal number. There are conflicting views as to the correct procedure for determining the instar, or life stage, of a given individual. Weisz (1946) advocated expressing the stage of development by referring to the number of body segments present, whereas Heath (1924) maintained that the instars were best separated on the basis of structual differences, particularly the development and modifications of the various appendages. A more detailed description of the life history and bionomics was given by Relyrea (1937) and Kuenen (1939).

MATERIALS AND METHODS.—The larvae were hatched from excysted eggs purchased from a commercial aquarium stock supply company. Hatching was accomplished by placing the eggs in a small beaker containing 3.5% saline solution. All cultures were reared in shallow enamel trays. All attempts to utilize 2- or 3-liter flasks failed.

Repeated tests indicated a 3.5% salt solution to be the best culture medium, with 27.5° C. as the optimum temperature. Though eggs hatched faster at lower salt concentrations, the percentage of survival was considerably lower. After repeated experiments using table salt, Turk's Aquarium Salt, and ordinary rock or ice cream salt, the latter consistently proved by far the more favorable form for rearing the brine shrimp. A salt preparation encountered later gave still better results. It was recommended by Michael *et al.* (1956) and is composed of the following compounds in the ratios given (expressed in grams): NaCl, 30; $CaSO_4$, 2; $MgSO_4$, 3; $MgCl_2$, 8.5; KCl, 0.8, and $MgBr_2$, 0.1.

Distilled water was utilized throughout the testing period, both in bioassay tests and in culture media. In each test, 2,000 ml. of water and 70 grams of rock salt were used. No attempt was made accurately to counterbalance the effect of evaporation. This, however, was not detrimental to the brine shrimp, but rather beneficial since they grew faster and attained a greater size at slightly higher salt concentrations, but hatched more rapidly at lower salt concentrations.

Among the materials tested as possible food sources were "Gaines Dog Food," "Pablum," "Fleischman's Yeast," "baker's yeast," and a commercial product called "Food for Brine Shrimp." "Gaines Dog Food" and "Pablum" were completely unsuitable as a food source, though the remaining three gave satisfactory results. "Food for Brine Shrimp" consistently appeared to be the better food source and was consequently utilized in rearing all cultures from which the specimens were used in bioassay work. The typical amount used was 150 milligrams for normal-sized populations of from 400 to 600 individuals. This amount was added at 24-hour intervals.

The number of eggs used was determined by experience, the viability varying with different vials of eggs, as well as with different forms and concentrations of salt. Excess mauplii were easily removed with an eye dropper since they normally congregated in the corners of the culture trays.

Tests were conducted on adults and various instars of the brine shrimp to determine the stage best suited for bioassay work. Those instars utilized were the 3rd, 5th, 7th, 9th, 11th and adults. Individuals in the first and second instars were considered too small to be practical for mass testing.

The saline solution used in bioassay tests was a 4% solution of distilled water and rock salt. It was purposely made more saline than the initial rearing medium to equate the change in concentration as a result of evaporation.

The test material was applied in all cases from an acetone solution. The test solutions of DDT, parathion, and dieldrin were prepared in the usual manner. Checks of acetone in saline solution at the highest concentration used in tests and 4% saline solution alone were used in conjunction with the compounds.

In order to eliminate as much variability as possible and to provide dosage-mortality curves which would be representative of the insecticides, the tests were run on each of 6 days using the same concentrations and approximately the same number of brine shrimp. All tests were conducted in 4-oz. jars, using 100 ml. of saline solution per jar. Each insecticide concentration was replicated six times and a total of five concentraions, with the exception of four for the adults, were used for each test. These concentrations were equally spaced logarithmically, each being double that below. Approximately 30 brine shrimp were used for each insecticide concentration to provide a larger population for testing so as to give a more representative indication of the toxicity of the compounds to the brine shrimp. These were divided nearly equally in three jars for ease of counting. Instars were determined with the aid of Heath's (1924) descriptions.

All mortality counts were made with a machine specifically designed for that purpose. It was constructed of plywood, measuring 8 inches in width, $8\frac{1}{4}$ inches in height, and 14 inches in length. The inner surface of the demountable top was equipped with a Wratten Safelight Filter measuring $5\frac{1}{4}$ inches in diameter and placed in the inner surface of the top. This was chosen specifically for overcoming the effects of intense light on photophobic persons, as well as to make the brine shrimp more conspicuous by providing a colored background. A 100-watt incandescent bulb was used throughout the testing period. A small electric fan, having a blade diameter of 5 inches was mounted at the rear of the box. This circulated air from a hole 4 inches in diameter at the rear of the box

Table 1.—LD_{50}'s of DDT, parathion and dieldrin to various instars and adults of *Artemia salina* (Leach).

Instar	Material in p.p.m.		
	DDT	Parathion	Dieldrin
Third	0.105	0.480	0.880
Fifth	.024	.320	.595
Seventh	.012	.462	.660
Ninth	.012	.390	.675
Eleventh	.028	.385	1.120
Adult	.142	.430	1.172

over the light and out a small hole 2 inches in diameter at the side of the box. Since the mortality counts were made in complete darkness, this latter opening also served as a source of light for recording the results. The light was mounted just anterior to the fan on a cross-piece of plywood which contained numerous holes for ventilative purposes. An aluminum plate was attached at the top of either side of the box, curved beneath the light, and served as a reflector.

In making mortality counts, it is of paramount importance that rigid standards and definitions be established. In executing those of brine shrimp, a combination of phototaxic reactions and deviations from normal swimming patterns through loss of musular coordination was utilized. This varied considerably among instars as well as insecticides, and corresponding changes were made to encompass these variations.

Mortality of the brine shrimp was determined after 5 hours' exposure to the test material in complete darkness. In all cases, the brine shrimp were allowed approximately 10 seconds after being exposed to light to exhibit some particular reaction.

Younger larvae were checked primarily by observation of deviations from normal swimming patterns, while their phototaxic reactions were of minor significance. Those immature forms which failed to exhibit characteristic movements were counted as dead. Normal individuals, particularly when placed in light following a period of complete darkness, manifested sweeping movements, rapidly covering a considerable distance, and, characteristically, swimming with the ventral surface facing the source of light. In test solutions, those individuals which continued to exhibit active movement but seemed attached to a particular location, were counted as dead. To be alive, an individual had to show not only muscular coordination, but had to travel a predetermined distance in a given time, comparable to that of the checks kept in water. Periodic observations of the checks were necessary to assure accuracy in determining mortality in tests conducted with earlier stages of the brine shrimp.

Older larvae, and adults especially, were observed primarily in relation to their phototaxic responses, with inability to exhibit muscular coordination being significant in some cases of border-line decisions. Adults, to be counted as unaffected, had to exhibit characteristic dorsoventral orientation to the source of light. Normal individuals on being exposed to light following a short period of darkness exhibit rapid postero-anterior spiral turns, essentially remaining in the same location while manifesting this reaction. Those failing to exhibit this response after 10 seconds had elapsed were considered dead. Individuals that faintly exhibited this response, yet manifested muscular incoordination, were also counted as dead.

Results.—The tests of each compound against a particular instar were made on each of 6 days and the results of the test summed and an average per cent mortality for each of the concentrations determined. The results were corrected for mortality in the checks by applying the formula devised by Abbott (1925). The mortality in the checks of acetone in saline solution, at the highest concentration used in tests and at 4% saline solution alone, was zero in all tests conducted on the 5th, 7th, 9th and 11th instars. Mortality was less than 1% in the adult stage and 5.2% in the check of acetone in saline solution and 4.8% in the saline solution in the third instar. The LD_{50}'s for the various compounds (table 1) were determined by plotting the results on logarithmic probability paper and fitting the line to the points by visual observation.

The susceptibility of the brine shrimp to DDT, parathion, and deldrin was: DDT > parathion > dieldrin. DDT and dieldrin exhibited similar trends in their toxicity to various stages of brine shrimp, but parathion showed no variation in toxicity to various stages of brine shrimp (fig. 1).

These results indicate that the brine shrimp is less sensitive to insecticides than larvae of *Aedes aegypti*

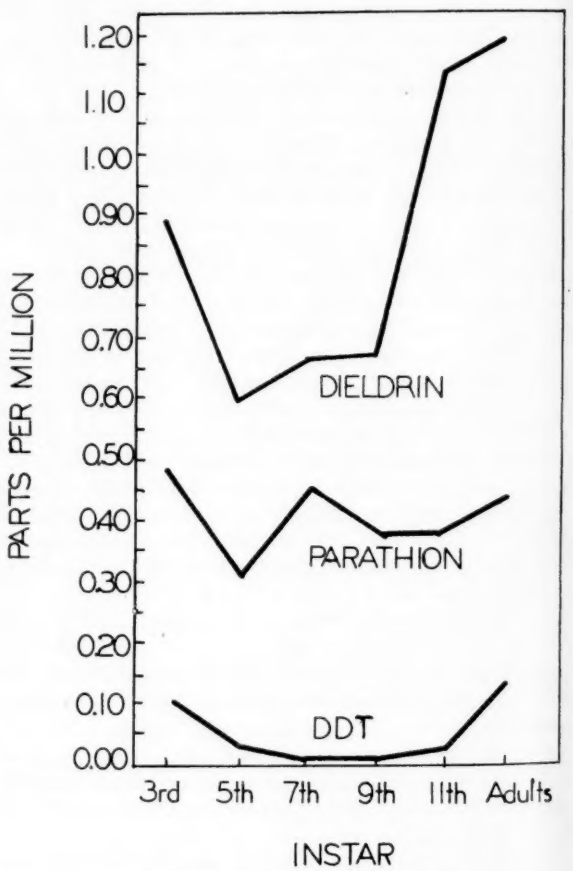


Fig. 1.—LD_{50}'s for DDT, parathion and dieldrin to various instar larvae and adults of brine shrimp.

(L.). Tests conducted by Banks (1955) at Clemson Agricultural College with mosquito larvae in an acetone standard solution of dieldrin gave an LD_{50} of 0.00077 p.p.m. This is significantly lower than that exhibited by the most sensitive instar of brine shrimp, the fifth, which gave an LD_{50} of 0.595 p.p.m. under similar circumstances. The difference would possibly be significantly lessened if the brine shrimp were checked after 24 hours exposure, as were the mosquito larvae, rather than 5 after hours.

REFERENCES CITED

Abbott, W. S. 1925. A method computing the effectiveness of an insecticide. Jour. Econ. Ent. **18**: 265–7.

Anonymous. 1955. Brine shrimp used in residue tests. Farm Chemicals **118**(9): 26.

Banks, William A. 1955. Bioassay of dieldrin in soil using larvae of *Aedes aegypti* (L.). Unpublished M. S. thesis, The Clemson Agricultural College, Clemson, South Carolina.

Heath, M. 1924. The external development of certain phyllopods. Jour. Morph. **38**: 453–83.

Kuenen, D. J. 1939. Systematical and physiological notes on the brine shrimp, *Artemia*. Arch. Neerland. Zool. **3**(4): 365–449.

Lochhead, John H. 1941. *Artemia*, the "brine shrimp." Turtox News **19**(2): 41–5.

Michael, A. S., C. G. Thompson, and M. Abramovitz. 1956. *Artemia salina* as a test organism for bioassay. Science **123**(3194): 464.

Pennak, Robert W. 1953. Fresh-water invertebrates of the United States. New York: The Ronald Press Co., p. 769.

Relyrea, G. M. 1937. The brine shrimp of Great Salt Lake. Amer. Nat. **71**: 612–6.

Weisz, P. B. 1946. The space-time pattern of segment formation in *Artemia salina*. Biol. Bull. **91**: 119–40.

Effectiveness of Several Insecticides against the Boll Weevil, Bollworm, and Cotton Leafworm[1]

R. L. WALKER, A. R. HOPKINS, and R. E. FYE, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

ABSTRACT

In small-plot experiments at Florence, S. C., against the boll weevil (*Anthonomus grandis* Boh.), Guthion-DDT, Thiodan, methyl parathion-DDT, EPN-DDT, and endrin dusts gave comparable control and yields. Sprays and dusts of Guthion and endrin and a spray of Dipterex were equally effective. Guthion-DDT spray gave significantly better control and yield than toxaphene and heptachlor-DDT sprays. Sprays of Guthion-DDT, Dipterex-DDT, methyl parathion-DDT, EPN-DDT, and endrin gave comparable control and yields. Dusts of Sevin, malathion, Guthion-DDT, and endrin gave comparable control. Sprays and dusts of Guthion, malathion, and endrin were equally effective. In a spray malathion was more effective at 2 pounds per acre than at 1 pound. Malathion plus endrin was more effective than endrin alone. Sprays of Sevin, Thiodan, endrin, toxaphene with and without methyl parathion, and methyl parathion gave comparable control.

Against the bollworm (*Heliothis zea* (Boddie)) malathion dusts were ineffective. Against the cotton leafworm (*Alabama argillacea* (Hbn.)) methyl parathion, malathion, and Guthion were more effective than BHC, endrin, and toxaphene.

In field-cage experiments against the boll weevil, malathion and methyl parathion gave consistently high 24-hour mortalities. Combinations of malathion with endrin or toxaphene were usually more effective than endrin or toxaphene alone. Guthion was more effective than toxaphene and BHC. Thiodan was less effective than endrin. Sevin at 2 pounds per acre gave high 48-hour kills. Monsanto CP-7769 and Chipman R-6199 were comparatively ineffective. Malathion was residually more effective than endrin, methyl parathion, or Thiodan.

Small-plot field experiments were conducted at Florence, S. C., in 1956 and 1957 to determine the effectiveness of several insecticides against the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)) and in 1957 against the cotton leafworm (*Alabama argillacea* (Hbn.)). Also in 1957 field-cage experiments were conducted against the boll weevil.

SMALL-PLOT EXPERIMENTS AGAINST BOLL WEEVIL AND BOLLWORM.—In both years one experiment was conducted with dusts, one with dusts and sprays, and two with sprays. The plots were 0.1 to 0.3 acre in size and were arranged in randomized blocks with four to six replicates. Dusts were applied with rotary hand guns and sprays with either a tractor-mounted or a high-clearance self-propelled sprayer with three nozzles per row. All sprays were applied at the rate of 5 gallons per acre.

Boll weevil and bollworm infestation records were made at about weekly intervals by examining 25 squares selected at random on each of the four center rows of each plot. In 1956 bollworm infestations were extremely light, preventing a valid determination of the value of the insecticidal treatments against this cotton pest. In 1957 a medium infestation developed in one of the experiments, and the percentage of injured bolls was determined in August by examining 50 bolls similarly selected.

Results in 1956 against Boll Weevil (*Table 1*).—In experiment 1 dusts of Guthion-DDT, Thiodan, methyl parathion-DDT, EPN-DDT, and endrin were equally effective. These treatments gave comparable yields and excellent increases over the untreated check.

In experiment 2 dusts and sprays of Guthion and endrin and a spray of Dipterex gave comparable control and yields. Excellent increases in yield were obtained over the check.

In experiment 3 a spray of Guthion-DDT was more effective than toxaphene or heptachlor-DDT and gave a significantly higher yield. All treatments except toxa-

[1] Accepted for publication May 16, 1958.

[2] In cooperation with the South Carolina Agricultural Experiment Station.

Table 1.—Effectiveness of several insecticides applied as dusts and sprays against the boll weevil in small-plot experiments. Florence, S. C.

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Pounds of Toxicant per Acre	Per Cent of Punctured Squares	Pounds of Seed Cotton per Acre	
			Total	Gain over Check
1956 Experiments				
Experiment 1 (7 applications from June 15 to August 3, 4 plots)				
Dusts				
Guthion 2.5+DDT 5	0.38+0.77	11.7	2312	772
Thiodan 3	.37	13.8	2203	663
Methyl parathion 5+DDT 5	.83+ .83	14.6	2178	638
EPN 5+DDT 5	.59+ .59	13.7	2177	637
Endrin 2	.23	12.8	2341	801
Untreated check[a]	—	55.3	1540	—
L.S.D. at 5% level	—	—[b]	—[b]	—
Experiment 2 (8 applications from June 15 to August 3, 5 plots)				
Dusts				
Guthion 2.5	0.17	18.9	1981	526
Guthion .5	.45	16.0	2168	713
Endrin .2	.19	14.9	2349	894
Sprays				
Guthion 2	.25	13.6	1971	516
Guthion 2	.5	15.9	2121	666
Dipterex 50% SP	1	18.3	1965	510
Endrin 1.6	0.2	17.4	2194	739
Untreated check[a]	—	40.8	1455	—
L.S.D. at 5% level	—	—[b]	—[b]	—
Experiment 3 (7 applications from July 10 to August 6, 4 plots)				
Sprays				
BHC 1+DDT 2	0.3 +0.5	24.2	2033	375
Toxaphene 6	2	33.2	1873	215
Heptachlor 2+DDT 2	0.25+ .5	26.5	1870	212
Guthion 2+DDT 2	.25+ .5	14.5	2122	464
Untreated check	—	39.1	1658	—
L.S.D. at 5% level	—	10.8	212	—
at 1% level	—	15.2	366	—
Experiment 4 (6 applications from July 10 to August 6, 4 plots)				
Sprays				
Guthion 2+DDT 2	0.25+0.5	23.8	1496	226
Dipterex 50% SP+DDT 2	2 + .5	26.3	1381	111
Methyl parathion 2+DDT 2	0.5 + .5	22.3	1479	209
EPN 4+DDT 2	.5 + .5	20.0	1437	167
Endrin 1.6	.2	23.3	1484	214
Untreated check	—	48.1	1270	—
L.S.D. at 5% level	—	12.6	—[b]	—
at 1% level	—	17.5	—	—
1957 Experiments				
Experiment 5 (6 applications from June 19 to August 2, 6 plots)				
Dusts				
Sevin 10	0.93	16.3	2302	150
Sevin 20	1.85	16.9	2530	378
Malathion 10	0.96	16.2	2302	150
Malathion 20	1.5	18.2	1966	−186
Guthion 2.5+DDT 5	0.24+ .48	17.9	2415	263
Endrin 2	.16	16.4	2404	252
Untreated check	—	26.2	2152	—
L.S.D. at 5% level	—	—	265	—
at 1% level	—	—	357	—
Experiment 6 (7 applications from June 14 to August 2, 4 plots)				
Dusts				
Guthion 2.5	0.21	16.7	1628	407
Endrin 2	.15	18.7	1666	445
Malathion 1.0	.88	17.6	1452	231
Sprays				
Guthion 1.5	.25	14.2	1467	246
Endrin 1.6	.2	22.9	1638	417
Malathion 5	1	20.2	1352	131
Untreated check[a]	—	36.0	1221	—
L.S.D. at 5% level	—	—[b]	—[b]	—
Experiment 7 (10 applications from June 17 to August 2, 4 plots)				
Sprays				
Guthion	0.25	14.3	1780	1380
Endrin 1.6	.2	39.7	1755	1355
Malathion 5	1	28.6	1698	1298
Malathion 5	2	16.5	1796	1396
Endrin 1.6+Malathion 5	0.2 + .25	31.1	1823	1423
Endrin 1.6+Malathion 5	.2 + .5	27.2	1836	1436
Untreated check[a]	—	75.4	400	—
L.S.D. at 5% level	—	8.9	—[b]	—
at 1% level	—	12.3	—	—
Experiment 8 (8 applications from June 14 to August 1, 5 plots)				
Sprays				
Sevin 1	1	15.3	—	
Sevin 1	2	15.9	—	
Thiodan 2	0.4	14.2	—	
Endrin 1.6	.2	15.4	—	
Methyl parathion 2	.5	13.0	—	
Toxaphene 6+methyl parathion	2 +0.3	15.8	—	
Toxaphene 6	2	20.0	—	
Untreated check	—	29.8	—	
L.S.D. at 5% level	—	9.0	—	
at 1% level	—	12.1	—	

[a] Untreated check located outside experimental area.
[b] Not significant according to the *F* test.

phene had significantly lower seasonal infestations than the check. Guthion-DDT and BHC-DDT gave yields that were highly significant compared with the check.

In experiment 4 sprays of Guthion-DDT, Dipterex-DDT, methyl parathion-DDT, EPN-DDT, and endrin were equally effective, each affording highly significant control. All treatments gave increases in yield over the check, but the differences were not significant.

Results in 1957 against Boll Weevil and Bollworm (Table 1).—In experiment 5 dusts of Sevin (1-naphthyl-N-methyl carbamate), malathion, Guthion-DDT, and endrin afforded lower seasonal boll weevil infestations than the untreated check, but none gave significant control. A 20% Sevin dust gave a significant increase in yield over the check. All treatments except malathion afforded excellent bollworm control. Malathion dusts at 10 and 20% strengths afforded no control.

In experiment 6 dusts and sprays of Guthion, endrin, and malathion afforded comparable seasonal boll weevil control and yields.

In experiment 7 malathion at 2 pounds and Guthion gave significantly better boll weevil control than malathion at 1 pound, endrin, or endrin plus malathion. Endrin with 0.5 pound of malathion gave control that was highly significant over that obtained with endrin alone. All treatments gave excellent increases in yield over the untreated check. There was no significant difference in yield between the insecticidal treatments.

In experiment 8 sprays of Sevin, Thiodan, endrin, toxaphene alone and with methyl parathion, and methyl parathion were equally effective against the boll weevil. Yields were not obtained owing to lack of uniformity in soil fertility and a heavy nematode infection in part of the experimental area.

EXPERIMENT AGAINST COTTON LEAFWORM.—Twenty cotton plants were selected at random on the two center rows of single plots and tagged. Five late-instar leafworms were placed on each tagged plant immediately prior to spraying. Sprays were applied with a high-clearance self-propelled sprayer with three nozzles per row at the rate of 5 gallons per acre. The reductions in population after 24 hours were as follows:

Insecticide	*Pounds per Acre*	*Per Cent Reduction*
Methyl parathion	0.5	93
Malathion	.5	86
Guthion	.25	83
BHC (gamma)	.3	61
Endrin	.2	58
Toxaphene	2	56

The organic-phosphorus insecticides afforded greater reductions than the chlorinated hydrocarbon insecticides.

FIELD-CAGE EXPERIMENTS WITH BOLL WEEVILS.—Square-reared weevils (2 to 3 days old) from the same field were used in all the experiments except experiment

Table 2.—Per cent boll weevil mortalities at 24 hours in field-cage experiments. Florence, S. C.

Insecticide (Pounds per Acre)	Experiment No. 1 (June 24)	2 (Aug. 15)	3 (Aug. 21, A.M.)	4 (Aug. 21, P.M.)
Malathion 0.5	80	—	—	—
Malathion 1	88	—	100	81
Malathion 2	96	—	96	100
Endrin 0.2	44	—	100	100
Endrin 0.2+malathion 0.25	88	—	—	—
Endrin .2+malathion 0.5	100	—	—	—
Guthion 0.25	100	—	—	—
BHC (gamma) 0.3	40	—	—	—
Sevin 2	32	—	—	—
Methyl parathion 0.5	—	—	100	100
Thiodan 0.4	—	—	82	80
Bayer 25141, 0.25	—	86	—	—
Untreated check	12	5	8	0

Table 4.—Per cent boll weevil mortalities at 24 hours when exposed to insecticides 1 day after treatment.

Insecticide (Pounds per Acre)	Experiment 10 (June 25)	Experiment 11 (Aug. 7)
Malathion 1	—	78
Malathion 2	96	94
Methyl parathion 0.5	—	42
Guthion 0.25	44	—
Endrin 0.2	4	46
Endrin 0.2+malathion 0.5	48	—
Thiodan 0.4	—	30
Untreated check	0	4

5, in which overwintering weevils were used. Single plots of cotton were sprayed, and immediately after the spraying weevils were caged on plant terminals. The cages were half-pint ice-cream cartons in which the tops and bottoms had been replaced with wire screen. Sprays were applied with a high-clearance self-propelled sprayer with three nozzles per row at the rate of 5 gallons per acre. Five cages of five weevils each were used per treatment except in experiment 9, where 10 cages of five weevils each were used. Mortalities were determined at the end of 24 hours in experiments 1–4, and at 24 and 48 hours in experiments 5–9. In experiments 10 and 11 residual effectiveness was determined by caging weevils 24 hours after treatment.

The results of these experiments are given in tables 2–4. In experiment 1 malathion at all three dosages and Guthion afforded kill superior to that of endrin, BHC, and Sevin. The addition of malathion to endrin greatly increased the kill over endrin alone.

In experiment 2 Bayer 25141 (*O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothioate) afforded a high kill. In experiments 3 and 4 malathion, endrin, and methyl parathion gave excellent kills and were slightly superior to Thiodan.

In experiment 5 Guthion, malathion, and methyl parathion gave 100% kill within 24 hours. Endrin and toxaphene gave considerably lower kill through 48 hours. The addition of 0.5 pound of malathion to endrin or toxaphene resulted in considerably increased kill over each alone.

In experiment 6 malathion at all dosages and endrin in combination with malathion at 0.5 pound afforded higher kills than endrin alone. Methyl parathion gave 100% kill within 24 hours. Monsanto CP-7769 (hexaethyl (ethylthiomethylidine) triphosphonate) gave a 48-hour kill comparable to endrin.

In experiment 7 malathion at 1 and 2 pounds, Sevin at 2 pounds, methyl parathion, and Guthion all afforded high kill at 48 hours. The addition of 0.25 or 0.5 pound of malathion to endrin or toxaphene resulted in considerably higher kills than were obtained with either alone. Monsanto CP-7769, Chipman R-6199, BHC, and 1 pound of Sevin gave low 48-hour mortalities.

In experiment 8 endrin gave 100% kill at 24 hours compared with 36% in experiment 7 conducted 14 days earlier. Topical-application tests with boll weevils from the same source showed greater susceptibility to endrin at the time of experiment 8, indicating a seasonal variation in susceptibility to endrin. In this experiment a lower kill was obtained with combinations of endrin and malathion than with endrin alone. However, the addition of malathion to toxaphene increased the kill over toxaphene alone. Malathion at all dosages, methyl parathion, and Guthion gave excellent kill. Sevin at 2 pounds af-

Table 3.—Per cent boll weevil mortalities in field-cage experiments.[a] Florence, S. C.

Insecticide (Pounds per Acre)	Experiment No. 5 (June 12)	6 (July 1)	7 (July 16)	8 (July 30)	9 (July 31)
Malathion 0.5	—	84, 88	64, 64	88, 92	—
Malathion 1	—	80, 84	68, 88	96, 100	92, 96
Malathion 2	100,	88, 92	100,	100,	96, 100
Endrin 0.2	52, 64	43, 60	36, 54	100,	76, 96
Endrin 0.2+malathion 0.25	—	—	56, 72	92, 92	—
Endrin 0.2+malathion 0.5	96, 100	72, 83	88, 92	59, 84	—
Toxaphene 2	32, 56	—	24, 52	42, 80	—
Toxaphene 2+malathion 0.25	—	—	72, 80	84, 96	—
Toxaphene 2+malathion 0.5	88, 92	—	44, 76	92, 92	—
Methyl parathion 0.5	100,	100,	92, 100	100,	100,
Monsanto CP-7769, 0.25	—	24, 56	16, 24	16, 32	—
Sevin 1	—	—	20, 43	44, 60	—
Sevin 2	—	—	60, 88	72, 96	—
Chipman R-6199, 0.4	—	—	26, 38	56, 72	—
BHC (gamma), 0.3	—	—	8, 9	21, 40	—
Guthion 0.25	100,	—	69, 89	84, 96	—
Thiodan 0.4	—	—			25, 43
Untreated check	0, 0	0, 0	0, 4	4, 20	4, 24

[a] First figure is mortality at 24 hours, second figure at 48 hours.

forded a high 48-hour kill. Monsanto CP-7769 and BHC resulted in low mortalities. Chipman R-6199 afforded a higher kill than BHC.

In experiment 9 malathion at 1 and 2 pounds and methyl parathion gave the highest 24-hour mortalities, but at 48 hours endrin gave comparable kill. The kill with Thiodan was much lower.

In experiment 10 malathion at 2 pounds showed outstanding residual effectiveness, being much superior to Guthion and a combination of endrin and malathion. Endrin was virtually ineffective.

In experiment 11 malathion at 2 pounds was also outstanding, giving considerably higher kill than methyl parathion, endrin, or Thiodan.

Concerning the Number of Times Queen Bees Mate[1]

STEPHEN TABER, III, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*,[2] and JAMES WENDEL, *Department of Mathematics, University of Michigan*

ABSTRACT

Four sets of mating data from different geographical locations and different strains of honey bees (*Apis mellifera* L.) obtained by three independent researchers are presented and discussed. One of these is new, the others have been presented before. The author's method of analysis is reviewed.

Two methods of analyses are presented, binomial and Poisson. The binomial aims to correct an error presented by Taber (1954) and Peer (1956) by a more complete definition of the mathematical model. The concept that matings may follow a Poisson-type distribution is discussed and analyzed.

The two methods of analysis indicate that the usual number of drone honey bees that meet and leave sperms with a queen is between seven and ten.

During recent years considerable evidence has been produced that queen bees (*Apis mellifera* L.) mate a number of times before they commence egg laying. Taber (1954) and Peer (1956) have presented mathematical analyses of progeny proportions indicating that the number of drones contributing sperm to a queen's spermatheca is greater than was previously thought, Triasko (1950, 1955) and Woyke (1955) have compared the relative volume of sperm from queens returning from mating flights. Ruttner (1955) gives a review of literature on queen mating.

The present paper aims to correct a theoretical error in Taber's and Peer's papers in using the binomial distribution for analysis. This error is one of mathematical definition in describing the probability model. All queens were considered to have mated an average number of times. This paper also presents the same data on the assumption that matings of queens and drones follow the Poisson distribution. Data are compared and summarized from studies from three geographical regions.

All data were obtained in essentially the same way; that is, a genetic recessive cordovan mutation was used to determine the number of matings. Cordovan queens mated to normal drones give normal progeny; cordovan queens mated to cordovan drones give cordovan progeny; cordovan queens mated to both cordovan and normal drones give cordovan and normal progeny.

Peer's data (Algonquin Provincial Park) were obtained at an isolated location where the drone population was under complete control. These data are probably the most reliable, since the colonies were supplied with queens heterozygous for the cordovan gene and theoretically should have produced equal numbers of normal and cordovan drones flying at all times. Peer estimated the drone population for his Ottawa data to be 60% normal and 40% cordovan. From his mating data Taber estimated the drone population at 33% cordovan and 67% normal. Data obtained by Cale[3] in an apiary supplied with all cordovan stock indicate a large proportion of normal drones flying in the vicinity of the mating location. The population of drones is estimated by the writer to be approximately 50% normal and 50% cordovan. Table 1 shows the data obtained by these researchers and the localities in which they worked.

Table 1.—Mating data from four studies of cordovan queens.

Investigator	Location	Drone Population		Queens with Progeny of Both Forms	Progeny	
		Normal	Cordovan		All Normal	All Cordovan
Cale	Illinois	50	50	241	3	6
Peer[a]	Algonquin Park	50	50	162	1	1
	Ottawa	60	40	293	9	1
Taber	Louisiana	67	33	171	13	0

[a] Drone populations theoretically reliable; others estimated from mating data.

On the basis of the data reported in table 1 it is impossible to distinguish the type of distribution of matings by a group of queens. However, some data have been presented indicating more nearly a Poisson distribution than any other. Roberts (1944) found 50% of the queens in his experiment returned with a mating sign once and 50% twice. His work also showed a decreasing number of queens flying each time up to five flights. Taber (1954) showed that a mating sign does not always indicate receipt of sperm by the queen.

The experimental results in table 1 can be viewed as the outcome of many replications of the following idealized two-stage experiment:

First Stage.—A positive integer N is selected at random from a population of integers in which the various integers n are present in the proportions p_n. Thus, the chance that the integer "one" is chosen, in symbols Pr $(N=1)$, will be p_1; similarly Pr $(N=2)=p_2$, and in general Pr $(N=n)=p_n$. More explicit assumptions about the p_n's will be specified presently.

[1] Accepted for publication May 19, 1958.

[2] In cooperation with Louisiana State University.

[3] G. H. Cale, Jr., of the staff of the American Bee Journal, kindly gave permission to use his unpublished data.

Second Stage.—When the result N = n has been determined, a coin having a probability p_1, of heads occurring, is tossed n times, so that the number of heads obtained follows the classical binomial law. As in flipping coins, the only information actually available to the observer is which one of the following events has occurred: (a) all "heads" cordovan, (b) all "tails" normal, or (c) both "heads" cordovan and "tails" normal. He does not know *N*, or even the distribution (p_n), nor is he able to observe N. His problem is to estimate the mean value or expectation of this random quantity, i.e., $E(N) = \overline{N} = \Sigma n p_n$, from the results of a large number of independent repetitions of the experiment.

In the above idealization each replication corresponds to the behavior of an individual queen, *N* denotes the number of mating encounters, and p the proportion of normal drones in the (mating) environment. The experimenter ultimately might like to determine the actual distribution of the number N, but for the moment he will settle for an estimate of its mean, $\overline{N}$. Even to estimate this relatively simple quantity it appears necessary to make explicit assumptions about the form of the distribution. This is done in two alternative ways here, binomial and Poisson, defined as follows:

Binomial Assumption.—Given a positive integer *k* and positive numbers *r* and *s* such that r+s=1, we assume that

$$P_n = \Pr(N = n) = C^k_{n-1} r^{n-1} s^{k-n+1} \text{ for}$$

$$n = 1, 2, \cdots, k+1 (C^k_{n-1} = \text{binomial coefficient})$$

$p_n = 0$ for all other values; then the mean value of *N*, $\overline{N}$ is given by

$$\overline{N} = kr + 1$$

and the parameters k and r will have to be estimated from the observations. In this article we assume in advance that $r = \frac{1}{2}$, so that $s = \frac{1}{2}$ also and only k has to be determined. The formulas above reduce to

$$p_n = (1/2^k) C^k_{n-1}, \qquad \overline{N} = (1/2)k + 1$$

Poisson Assumption.—Given a positive parameter λ, we assume that

$$p_n = e^{-\lambda} n-1 \lambda/(n-1)!, \qquad n = 1, 2, \cdots$$

where e is the base of the natural logarithms. Then $\overline{N} = \lambda + 1$, and λ must be estimated.

These alternatives describe the assumptions relative to the first stage of the idealized experiment. For the second stage the familiar binomial law is assumed to hold in both Cases, so that

Pr (all normal, given that n matings have occurred)

$$= \Pr(\text{all normal} \mid N = n) = p^n, p = \text{proportion of normal drones,}$$

and similarly

$$\Pr(\text{all cordovan} \mid N = n) = q^n, q = 1 - p \text{ proportion of cordovans;}$$

then

$$\Pr(\text{both} \mid N = n) = 1 - p^n - q^n.$$

Table 2.—Analysis of binomial distribution of mating data obtained from four studies of cordovan queens..[a]

Investigator	Q	k	N	Number of Queens with All Normal and All Cordovan Progeny Expected	Actual
Cale	250	11	6.5	10.5586	9
		12	7	7.9175	
Peer					
Algonquin Park	164	14	8	2.9216	2
		15	8.5	2.1915	
		16	9	1.6438	
Ottawa	303	13	7.5	11.4478	10
		14	8	9.0246	
Taber	184	11	6.5	17.2176	13
		12	7	14.2273	
		13	7.5	11.7783	

[a] Probability of n matings—$1/2^k C^k_{r-1}$

Then for the combined two-stage experiment the values are

$$\Pr(\text{all normal}) = \Sigma \Pr(\text{all normal} \mid N = n) \Pr(N = n)$$

with a similar expression for cordovans; under the two alternative models the results are as follows:

Binomial assumption.—

$$\Pr(\text{all normal}) = p[(1+p)/2]^k, \Pr[\text{all cordovan}] = q[(1+q)/2]^k,$$

$$\Pr[\text{all N or all C}] = \text{total.}$$

Expected number of queens with progeny all of one kind = Q times total, where Q = total number of queens flown. The values of these quantities are listed in table 2 for various choices of K; the values of p and q are those under the headings N and C in Table 1. $\overline{N} = (\frac{1}{2})k + 1$.

Poisson assumption.—

$$\Pr(\text{all normal}) = pe^{-\lambda q}, \quad \Pr(\text{all cordovan}) = qe^{-\lambda p},$$

expected number of queens with progeny all of one kind = Q times total of these, $\overline{N} = \lambda + 1$. Values are given in table 3.

The method of maximum likelihood can be used to derive a formula (assuming Poisson's distribution) that gives a direct answer where the drone populations of the cordovan, mutant, and normal or wild type are equal. The basis for the detailed derivation of the formula and calculation using Peer's Algonquin Park data follows: Assume that the proportions p_n follow the modified Poisson law, in which n ranges over all positive integers, n = 1, 2, . . . (In the ordinary form of the Poisson distribution n appears in place of n−1 in the expression of p_n and the values of n start at 0 rather than at 1. But in the problem considered here n represents the number of mating encounters, and n = 0 is without meaning.) Assume also that $p = q = \frac{1}{2}$.

Under these assumptions the mean value, $\overline{N}$, of the number of mating encounters works out to be

$$N = \lambda + 1.$$

Table 3.—Analysis by Poisson distribution of mating data obtained from four studies of cordovan queens.

Investigator	Assumed Average N	Number of Queens with All Normal and All Cordovan Progeny Expected	Actual
Cale	7	7.549	9
	7.5	9.693	
Peer	9.5	2.3384	2
Algonquin Park	9.8	2.0133	
	10	1.8217	
Ottawa	8.5	10.398	10
	9	8.6015	
Taber	7.5	14.8568	13
	8	12.4714	

Thus we wish to estimate the unknown quantity λ on the basis of the following observations:

c = number of queens producing progeny all mutant (cordovan)
w = number of queens producing progeny all wild type (normal)
b = number of queens producing progeny of both kinds.

The method of maximum likelihood consists in writing down the probability of the observed result and choosing the value of λ that maximizes it. The probability of the result c, w, b, is proportional to

$$p^w e^{-\lambda q w} q^c e^{-\lambda p c} (1 - p e^{-\lambda q} - q e^{\lambda p})^b$$

for general p and q, or

$$(1/2)^{w+c} e^{-\lambda(w+c)/2} (1 - e^{-\lambda/2})^b$$

for $p = q = \frac{1}{2}$. The value of λ that maximizes this, found by setting the derivative equal to zero and solving for λ, is

$$2 \log \frac{b + w + c}{w + c}$$

(natural logarithm).

For Peer's data we have $c = w = 1$, $b = 162$, and therefore the maximum likelihood estimate of λ is

$$= 2 \log 164/2$$
$$= 2 \log 82$$
$$= 8.8$$

Therefore, the estimate of $\bar{N}$ is $8.8 + 1 = 9.8$

When the four sets of data and the two methods of analysis are compared, it will be seen that the Poisson distribution gives an estimate of about one more mating per queen than the binomial distribution. Since the mating distribution is not known, the reader can take his choice, for it is apparent that either method is applicable. It is also obvious that other methods and distributions may be applicable. Is it possible, then, that most queens are mating only two or three times? Yes, and for the same reason and with the same probabilities with which one can toss three coins 901 times and obtain either just heads or just tails 34 times; the unlikely is not impossible.

There seems to be little reason for not accepting Robert's (1944) experimental setup, by which he determined that 50% of the queens make one mating flight and 50% two flights, as approximating natural conditions. If under these conditions the combined average number of drones that they meet ranges from seven to ten, then for each mating flight a queen would mate with five to seven drones.

Taber's (1954) data on single mating flights do not disagree with these figures, but the number of queens involved is too small for a detailed comparison to be made. They also agree substantially with those of Woyke (1955).

Of perhaps more importance than the average number of matings per queen is the range in number of matings to be expected in a particular queen; that is, any one queen would be expected to have mated with 1 to 20 drones. Hence we should expect sometime to find a queen that has mated only once and another mated as many as 20 times. However, proof of the exact number of matings is probably of less importance than information as to the distance and direction of the nuptial flight and how queens and drones find each other.

The number of sexually mature drones flying may affect the mating distribution more than is supposed. It seems likely, however, that increasing the ratio of drones to virgin queens flying cannot influence the mating distribution beyond a certain point. Certainly a tremendous excess of drones flying should tend to increase the number of matings. Most workers have assumed that the number of drones available for matings is adequate.

In the presentation of the two possible mating distributions, mating is considered as receipt of sperms from a drone. These are termed "discrete distributions." It is probable that varying numbers of sperm are received from each drone. If mating is defined in such a way that the maximum number of sperm cells received in the spermatheca from one drone equals 1 and that from drones contributing less than this amount is a fraction of 1, then there would exist a continuous distribution. As a numerical example, suppose a queen could receive a maximum of one million sperm cells from one drone, yet she met with eight drones and received a total of 5.5 million sperm cells. This illustrates still another problem of analysis, though the experimental method seems rather difficult to approach.

To state, as Peer does, that "The use of the binomial presupposes that all queens mate the same number of times" is to assume that those queens that mate one more than the average contribute as much to the data as the queens that mate one less than the average. This is an erroneous assumption as the figures presented illustrate.

References Cited

Alber, M., R. Jordan, F. Ruttner, and H. Ruttner. 1955. Von der Paarung der Honigbiene. Zeitschr. f. Bienenforsch. **3**(1): 1–28.

Peer, D. F. 1956. Multiple mating of queen honey bees. Jour. Econ. Ent. **49**(6): 741–3.

Roberts, W. C. 1944. Multiple mating of queen bees proved by progeny and flight tests. Gleanings in Bee Culture **72**(6): 255–9, 303.

Ruttner, F. 1956. The mating of the honeybee. Bee World **37**: 3–15.

Taber, S. 1954. The frequency of multiple mating of queen honey bees. Jour. Econ. Ent. 47(6): 995–8.
Tryasko, V. V. 1951. Signs indicating the mating of queens. Pchelovodstvo (11): 25–31. (In Russian)
Tryasko, V. V. 1956. Repeated and multiple mating of queens. Pchelovodstvo 33: 43–50. (In Russian)
Woyke, J. 1955. Multiple mating of the honeybee queen (*Apis mellifica* L.) in one nuptial flight. Bull. Acad. Polonaise Sci. Cl. 2 3(5): 175–80.

The Efficiency of Systemic Insecticides in the Control of the Smaller European Elm Bark Beetle[1]

A. F. AL-AZAWI and J. E. CASIDA,[2] *Department of Entomology, University of Wisconsin, Madison*

ABSTRACT

Following trunk implantation into mature elm trees, demeton, dimefox, Thimet and Chipman R-6199 were readily translocated into the foliage and persisted many weeks. Only Chipman R-6199 at a dosage of 8 grams per tree gave marked control of *Scolytus multistriatus* (Marsh.) In laboratory tests for toxicity of various systemics to this elm bark beetle, Chipman R-6199 was inferior to other organophosphates. The efficiency in the intact tree may be associated with localization in cortical tissues. In no case was a systemic as effective as DDT in beetle control and the prevention of beetle feeding.

Several systemic insecticides will control insects attacking deciduous and coniferous trees when applied to the soil and bark or implanted into the trunk (Kinghorn 1955, Ripper 1957, Nagel 1957, Central States Forest Expt. Sta. 1957, Giese *et al.* 1958). No attempts at systemic control of elm bark beetles have been reported. The Dutch elm disease fungus, *Ceratocystis ulmi* (Buisman) Moreau, is transmitted from diseased to healthy trees by the elm bark beetles, *Scolytus multistriatus* (Marsh.) and *Hylurgopinus rufipes* (Eichh.). The only known method to control the causal organism is to control the insect vectors.

Preliminary studies were conducted on the translocation of organophosphate systemic insecticides in elm trees (*Ulmus americana* L.) following trunk implantation and their efficiency in controlling the smaller European elm bark beetle, *S. multistriatus*.

METHODS AND RESULTS.—*Toxicity of various organophosphate systemic insecticides to Scolytus multistriatus.* The toxicity of several organophosphates was compared with that of DDT. Adult beetles were obtained by confining infested logs in large metal cans with small glass jars mounted on the sides of the cans. The emerging phototropic beetles were easily collected from the jars. The insecticides were dissolved in acetone and applied to the cut ends of fresh $\frac{1}{2}$-inch diameter elm twig sections weighing 3.8 to 4.5 grams each. The parts per million of insecticide were calculated on the total weight of the section although the insecticide was only applied to the cut surfaces. After the acetone had evaporated, two treated sections were placed in a vial along with five to 10 beetles and the mortality observed after 48 hours. Two to six replicates were run at each dosage to establish the approximate LD_{50} value for each insecticide. Under these conditions the 48-hour LD_{50} for p,p'-DDT was between 3 and 10 p.p.m. Thimet, dimefox and Bayer 19639 were more active with LD_{50}s in the range of 1 to 3 p.p.m. Demeton, Am. Cyanamid 12880 (*O,O*-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate) and Am. Cyanamid 18706 (*O,O*-dimethyl S-(N-ethylcarbamoylmethyl) phosphorodithioate) were of lower toxicity (LD_{50}s of 30 to 100 p.p.m.). The least toxic materials (LD_{50} of 300 to 1000 p.p.m.) were *O,O*-diethyl S-(2-diethylamino)ethyl phosphorothioate as the free base, the hydrogen oxalate salt (Chipman R-6199) and the *p*-toluenesulfonate salt (Chipman R-6200).

Toxicity of elm branches which had absorbed certain systemic insecticides to S. multistriatus.—Fresh elm branches of about 6-foot length and $\frac{3}{4}$-inch diameter were allowed to absorb solutions or emissions of Chipman R-6199, Thimet, dimefox and demeton. After each branch had absorbed 50 to 100 mg. actual insecticide, the basal portion was cut off and 30 to 60 beetles confined on the terminal portions which contained only the translocated systemic. Dimefox and demeton were most effective in killing *S. multistriatus* based on the 24- and 48-hour beetle mortality, and reduction in length of the beetle feeding scars compared with controls. Thimet was intermediate in effectiveness and Chipman R-6199 the least toxic.

Rate of uptake of systemics in mature elm trees following implantation treatments.—Two mature elm trees, 4 inches in breast height diameter and 40 feet in height, were treated by implantation with 8 grams of demeton for one tree and 4 grams of Chipman R-6199 for the other. Implantation was accomplished as previously described (Giese *et al.* 1958). Leaf samples were assayed for anticholinesterase agents (see footnote of table 2 for method) at 1, 2 and 3 days after treatment. With Chipman R-6199 high levels of anticholinesterase agents were present in the leaves at each time, and with demeton there was a progressive increase from 1 to 3 days. At 7 days after treatment with Chipman R-6199, leaves from each of 10 major branches on the tree were assayed for anticholinesterase agents. The amount of R-6199 in the foliage was found to vary by a factor of two to three times between branches.

Toxicity of branches to S. multistriatus and anticholinesterase agents in the foliage after implantation of systemic insecticides in mature elms.—In July 1957 mature elm trees were treated by implantation with Thimet, demeton,

[1] Accepted for publication May 19, 1958. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. The data were presented in part at the Fifth Annual Meeting of the Entomological Society of America in Memphis, Tennessee, December, 1957.

[2] The authors gratefully acknowledge the counsel and technical assistance of Dr. D. M. Norris, Jr., Department of Entomology, and Dr. E. B. Smalley, Department of Plant Pathology, University of Wisconsin; Mr. G. E. Hafstad, Wisconsin Department of Agriculture; and the skilled technical assistance of Miss Judy Dengler.

Table 1.—Mortality of *Scolytus multistriatus* feeding on branches of elm trees treated with Chipman R-6199.[a]

Days After Treatment	Per Cent Mortality[b] After		Per Cent Reduction in Length of Feeding Scars[c]
	24 Hours	48 Hours	
5	78	—	—
16	49	86	75
33	34	76	70
56	10	51	—
81	4	56	56
DDT[d]	84	98	99

[a] 2 gms. actual R-6199 implanted in each of four holes for a total of 8 gms. per 4-inch breast height diameter, 40-foot high elm tree.

[b] Uniform elm branch samples taken at various times after treatment were brought into the laboratory and placed in small cylindrical cages with from 11 to 100 beetles. Mortality counts were taken after 24 and 48 hours and corrected for control mortality by Abbott's formula (1925).

[c] The length of each feeding scar was measured after the beetles were confined with the branches for 48 hours. The feeding scar length per beetle from treated trees was compared with the length per beetle for untreated trees and expressed as per cent reduction due to treatment.

[d] DDT sprayed on dormant elm trees according to current recommendations for control of elm bark beetles. Branches sampled 3 days after DDT spray.

dimefox and Chipman R-6199 at 1 or 8 grams of actual insecticide per tree. Chipman R-6199 was used as a 50% aqueous solution while the other systemics were used as the technical grade product. Sixteen trees in all were insecticide-treated and an additional four water-treated trees served as controls. At several times after treatment twigs from these trees were brought to the laboratory and assayed immediately for toxicity to *S. multistriatus* (table 1). At 5 days after implantation, no significant beetle mortality occurred with any of the systemics at the 1-gram-per-tree dosage; therefore, only the 8-gram-per-tree dosages were assayed at later times. Leaf samples representative of the total foliage were also taken at various times after implantation, quick frozen and held at −10° C. until assayed for anticholinesterase agents (table 2). None of the systemics were phytotoxic at the 1-gram-per-tree level and only dimefox gave any indication of foliar "burning" at the 8-gram-per-tree dosage.

At all times after treatment of the trees, Chipman R-6199 was far superior to any other systemic and was the only one that gave significant beetle kill and reduction in the length of the feeding scars (table 1). However, all the systemics were translocated into the foliage and persisted for over 17 weeks. Little change in the level of Chipman R-6199 in the foliage occurred during the 17 weeks following treatment, but the results are less readily interpreted with the other systemics since they are metabolized within the plant to form more potent anticholinesterase agents (Casida 1956).

A DDT spray applied to dormant elm trees according to recommended procedures (Chambers 1957) was more effective in controlling *S. multistriatus* than any of the systemics at the dosages used. The DDT was much more effective than the Chipman R-6199 in minimizing the extent of bark beetle feeding before mortality, a factor which may be critical in relation to possible use in control of the Dutch elm disease.

Table 2.—Anticholinesterase agents in elm foliage after implantations of certain systemic insecticides at 8 grams per tree.

Weeks After Implantation	Milligrams Leaf Tissue for 50% ChE Inhibition[a]			
	Demeton	Dimefox	Chipman R-6199	Thimet
1	90	640	0.12	193
3	90	620	0.13	213
4	77	620	0.10	150
6	53	540	0.12	213
9	48	520	0.14	143
11	53	460	0.14	130
13	60	260	0.13	160
15	67	170	0.08	120
17	68	200	0.06	93

[a] The fresh weight of leaf in a tissue homogenate which when added to 5.0 ml. of 30% whole human blood will inhibit the blood cholinesterase activity 50% with 1 hour incubation at 37° C. prior to addition of substrate (method of Casida *et al.* 1956).

Discussion.—Chipman R-6199 was the least active chemical in the laboratory and the most active in the field for the control of the smaller European elm bark beetle. The efficiency of this systemic in beetle control was greatest shortly after treatment and declined progressively throughout the season. A high level of anticholinesterase agents, however, persisted throughout the season in the foliage. These combined observations indicate that Chipman R-6199 might be localized in the cortical tissues more than the other systemics studied. It is interesting to note that R-6199 was the only systemic studied which could form salts with plant acids which might aid in localization. Chipman R-6200 appears in high levels in certain cortical tissues after trunk implantation of cacao (Bowman & Casida 1958). Assay of the bark of elm trees treated with Chipman R-6199 also showed high levels of anticholinesterase agents.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Jour. Econ. Ent. **18:** 265–7.

Bowman, J. S., and J. E. Casida. 1958. Systemic insecticides for *Theobroma cacao* L., their translocation and persistence in foliage and residues in cacao beans. Jour. Econ. Ent. **51**(6): 773–80.

Casida, J. E. 1956. Metabolism of organophosphorus insecticides in relation to their antiesterase activity, stability and residual properties. Jour. Agric. Food Chem. **4:** 772–85.

Casida, J. E., P. E. Gatterdam, L. W. Getzin, Jr. and R. K. Chapman. 1956. Residual properties of the systemic insecticides *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate. Jour. Agric. Food Chem. **4:** 236–43.

Central States Forest Expt. Sta., Columbus, Ohio. 1957 Ann. Rept., mimeo, pp. 51.

Chambers, E. L. 1957. Dutch elm disease. Wisconsin State Dept. Agric., Spec. Bull. **62,** 4 pp.

Giese, R. L., D. M. Benjamin and J. E. Casida. 1958. Preliminary studies on trunk implantation of systemic insecticides in conifers. Jour. Econ. Ent. **51**(3): 400–1.

Kinghorn, J. M. 1955. Chemical control of the mountain pine beetle and Douglas-fir beetle. Jour. Econ. Ent. **48:** 501–4.

Nagel, R. H. 1957. Control of the European elm scale with systemic insecticides. Paper presented at the 5th annual meeting of the Ent. Soc. of Amer., Dec. 1957, Memphis, Tennessee.

Ripper, W. E. 1957. The status of systemic insecticides in pest control practices. Adv. in Pest Control Res. **1:** 305–52.

Screening Tests with Some Synthetic Compounds for Toxicity to House Flies When Applied as Space Sprays[1]

P. G. Piquett and W. A. Gersdorff, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

Abstract

The results of preliminary evaluation of 111 synthetic compounds when applied as space sprays for control of the house fly (*Musca domestica* L.) are reported. Eighteen compounds, all esters of chrysanthemumic acid or a closely related cyclopropanecarboxylic acid, were sufficiently toxic to warrant further study.

In the search for new insecticidal materials at the Beltsville, Maryland, laboratories, many compounds have been synthesized and tested as insect toxicants, synergists, repellents, or attractants. The results of preliminary evaluation of 111 compounds[2] as space sprays against the house fly (*Musca domestica* L.) are reported in this paper.

Because of the low solubility of some of the compounds in kerosene, all were prepared as solutions in 1:3 acetone-Deobase (refined kerosene) at a concentration of 20 mg. per milliliter. Mortality was determined in duplicate tests on laboratory-reared house flies by the Campbell turntable method. One hundred flies averaging 2 to 3 days in age were used in each test.

The materials were tested over a period of several months on many populations of flies often of different susceptibility. Therefore, the results are placed in groups according to mortalities caused by the standards, 1 and 2 mg. of pyrethrins per milliliter of the same solvent mixture. Since acetone affects toxicity (Gersdorff 1956), comparability of the tests was retained by keeping the acetone content in the sprays uniform.

The mortalities after 1 day are given below:

[1] Accepted for publication May 23, 1958.

[2] Synthesized by Yuh-Lin Chen and W. F. Barthel (1956) or by W. F. Barthel and B. H. Alexander (1957, 1958, and unpublished work).

Ent. No.	Name	Per Cent Mortality
	Pyrethrins 10–15% at 1 mg. and 30–35% at 2 mg.	
21,275	Benzene, 4-(1-butoxyethoxy)methyl-1,2-methylenedioxy-	1
21,289	3-(1-butoxyethoxy)methyl-1,2-dimethoxy-	3
21,291	3-[1-(2-chloroethoxy)ethoxy]methyl-1,2-dimethoxy-	3
21,292	3-(1-ethoxyethoxy)methyl-1,2-dimethoxy-	8
21,293	3-[1-(2-ethylhexyloxy)ethoxy]methyl-1,2-dimethoxy-	0
21,290	3-(1-isobutoxyethoxy)methyl-1,2-dimethoxy-	4
21,276	4-(1-isobutoxyethoxy)methyl-1,2-methylenedioxy-	1
21,538	Benzyl alcohol, *p*-(2-methylallyloxy)-, acetate	0
21,539	*p*-propoxy-, acetate	1
21,556	Chrysanthemumic acid, *p*-butoxybenzyl ester	0
21,285	3-(*p*-isopropylphenyl)-1-methylpropyl ester	1
21,286	3-(*p*-methoxyphenyl)-1-methylpropyl ester	2
21,531	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methylpropyl)-, *p*-ethoxybenzyl ester	0
21,253	*m*-Dioxane, 2-methyl-5-piperonyl-	6
21,540	Piperonal, 6-chloro-	1
	Pyrethrin 20–30% at 1 mg. and 56–65% at 2 mg.	
20,339	Acetic acid, α-isopropylpiperonyl ester	14
21,464	phenyl-, 6-bromopiperonyl ester	1
21,338	phenyl-, *p*-ethoxybenzyl ester	2
21,618	Benzene, 5-allyl-4-(1-[2-(2-ethoxyethoxy)ethoxy]ethoxy)-methyl-1,2-methylenedioxy-	5
20,986	1,2-dibromo-4,5-methylenedioxy-	1
21,503	4-[1-(2-chloroethoxy)ethoxy]methyl-1,2-dimethoxy-	0
21,576	4-(1-[2-(2-ethoxyethoxy)ethoxy]ethoxy)methyl-1,2-methylenedioxy-	0
21,619	4(1-[2-(2-ethoxyethoxy)ethoxy]ethoxy)methyl-1,2-methylenedioxy-5-propenyl-	2
21,612	4-[3-(1-[2-ethoxyethoxy)ethoxy]ethoxy)-2-methyl-propyl]-1,2-methylenedioxy-	16
21,502	4-(1-ethoxyethoxy)methyl-1,2-dimethoxy-	2
21,501	4-[1-(2-ethylhexyloxy)ethoxy]methyl-1,2-dimethoxy-	0
21,334	Benzoic acid, 2-allyloxy-3-ethoxy-, ethyl ester	2
21,427	Chrysanthemumic acid, *o*-allyloxybenzyl ester	88
21,422	*m*-allyloxybenzyl ester	88
21,573	*m-sec*-butoxybenzyl ester	62
21,578	*p-sec*-butoxybenzyl ester	0
21,339	*p*-butoxyphenethyl ester	2
21,423	*p-tert*-butylbenzyl ester	0
21,557	6-chloropiperonyl ester	100
21,424	*o*-ethoxybenzyl ester	14
21,340	*m*-ethoxybenzyl ester	82
21,425	*m*-isobutoxybenzyl ester	2
21,426	*m*-isopropoxybenzyl ester	97
20,337	α-isopropylpiperonyl ester	3
20,410	2,3-dimethoxybenzyl ester	75
21,577	*p*-(2-methylallyloxy)benzyl ester	0

Ent. No.	Name	Per Cent Mortality
21,572	*m*-methylbenzyl ester	42
20,274	piperonyl ester	96
21,621	Cyclopropanecarboxamide, *N,N*-diethyl-2-piperonyl-	13
21,574	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methylpropyl)-, 2,4-dimethylbenzyl ester	86
21,499	piperonyl ester	92
21,500	*m*-propoxybenzyl ester	5
20,292	*m*-Dioxane, 4-methyl-2-(3,4-methylenedioxyphenyl)-	5
20,386	4,6-dimethyl-2-(3,4-methylenedioxyphenyl)-	2
21,570	Ether, isobutyl phenyl	1
20,384	Piperonyl alcohol, α-pentyl-	2
20,356	Senecioic acid, allethrolonyl ester	2
20,934	Succinic acid, piperonyl-, dimethyl ester	0
	Pyrethrins 31–40% at 1 mg. and 66–75% at 2 mg.	
21,699	Acrylic acid, β-(3,4-methylenedioxyphenyl)-α, phenyl-, ethyl ester	1
21,732	methyl ester	2
21,733	Chrysanthemumic acid, *p*-allyloxybenzyl ester	2
21,341	*p*-ethoxyphenethyl ester	1
21,558	*p*-ethylbenzyl ester	100
21,564	*m*-(2-methylallyloxy)benzyl ester	69
21,563	2,5-dimethylbenzyl ester	40
21,559	*o*-methylbenzyl ester	67
21,342	*m*-propoxybenzyl ester	82
21,560	*p*-propoxybenzyl ester	1
21,353	*m*-Dioxane, 2-methyl-4-(3,4-methylenedioxyphenyl)-5-phenyl-	2
21,346	2,2-dimethyl-4-(3,4-methylenedioxyphenyl)-5-phenyl-	1
	Pyrethrins 51–65% at 1 mg. and 81–90% at 2 mg.	
20,104	Acetic acid, phenyl-, 2-methylpentyl ester	1
21,056	Acetonitrile, 3,4-methylenedioxyphenyl-	7
21,041	Anisole, *p*-(2-butenyloxy)-	2
21,257	Benzene, 4-(1-ethoxyethoxy)methyl-1,2-methylenedioxy-	0
21,256	4-[1-(2-ethylhexyloxy)ethoxy]methyl-1,2-methylenedioxy-	1
21,165	Benzothiazole, 2-(allylthio)-	1
21,166	2-(benzylthio)-	1
21,167	2-(propylthio)-	1
20,553	Benzyl alcohol, 3,4-dichloro-	12
20,493	*p*-chloro-acetate	9
21,160	2,4-dimethyl-, acetate	7
21,161	*p*-ethoxy-, acetate	2
21,169	4-Chromanone, 6-methoxy-2-methyl-	1
20,003	Chrysanthemumic acid, α-allylpiperonyl ester	0
20,248	benzyl ester	53
20,144	cinnamyl ester	3
20,422	cuminyl ester	96
21,036	3-cyclohexyl-2-methyl-4-oxo-2-cyclopenten-1-yl ester	67
21,171	*p*-ethoxybenzyl ester	2
20,272	α-ethylpiperonyl ester	1
21,173	*m*-methoxybenzyl ester	78
20,143	*p*-methoxybenzyl ester	48
21,170	2,4-dimethylbenzyl ester	100
21,172	Chrysanthemumic acid, 3,4-methylenedioxyphenethyl ester	11
20,244	4-(3,4-methylenedioxyphenyl)-*sec*-butyl ester	1
20,110	α-methylpiperonyl ester	70
20,411	α-(1,1-dimethylpropyl)piperonyl ester	97
20,412	α-pentylpiperonyl ester	4
20,091	α-propylpiperonyl ester	8
20,421	Chrysanthemummonocarboxamide, *N,N*-diethyl-	4
21,054	Cyclopropanecarboxylic acid, 2-methyl-3-(3,4-methylene-dioxyphenyl)-, methyl ester	3
21,055	2-piperonyl-, methyl ester	1
21,255	*m*-Dioxane, 2-ethyl-2-methyl-5-piperonyl-	0
21,254	2-ethyl-5-piperonyl-	0
20,182	1,3-Dioxolane, 4-methyl-2-(3,4-methylenedioxyphenyl)-	1
20,227	4,5-dimethyl-2-(3,4-methylenedioxyphenyl)-	2
21,138	Phenol, 2-allyl-6-methoxy-, acetate	3
21,042	2-(2-butenyl)-4-methoxy-	1
20,415	Piperonyl alcohol, formate	4
21,184	6-bromo-, acetate	4
21,057	α-(*p*-methylphenyl)-	2
21,038	α-(*o*-methylphenyl)-, acetate	2
21,039	α-(*p*-methylphenyl)-, acetate	4
20,484	Senecioic acid, α-(3-phenylpropyl)piperonyl ester	4
21,033	Succinic acid, 2-piperonyl-, 4-ethyl 1-isopropyl ester	3
20,219	*p*-Toluamide, *N,N*-diethyl-	2

Twenty-nine compounds showed some toxicity at the concentration tested. Further study is warranted with 18 because of toxicity or interest in the chemical structure, or both. They are esters of chrysanthemumic acid or a closely related cyclopropanecarboxylic acid. Their approximate ratings in comparison with pyrethrins (100%) are given below.

	Per Cent
Chrysanthemumic acid, 2,4-dimethylbenzyl ester	33[a]
6-chloropiperonyl ester	33[a]
p-ethylbenzyl ester	33[a]
piperonyl ester	33
m-isopropoxybenzyl ester	33
Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methyl-propyl)-, piperonyl ester	25
Chrysanthemumic acid, α-(1,1-dimethylpropyl)piperonyl ester	20
cuminyl ester	20
o-allyloxybenzyl ester	20
m-allyloxybenzyl ester	20
m-ethoxybenzyl ester	20
Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methyl-propyl)-, 2,4-dimethylbenzyl ester	20
Chrysanthemumic acid, 2,3-dimethoxybenzyl ester	15
m-propoxybenzyl ester	13
m-*sec*-butoxybenzyl ester	10
m-methoxybenzyl ester	10
m-(2-methylallyloxy)benzyl ester	10
o-methylbenzyl ester	10

[a] Possibly much greater, since the test spray caused 100% mortality.

References Cited

Barthel, W. F., and B. H. Alexander. 1957. Screening test of miscellaneous compounds containing the methylenedioxyphenyl structure. U. S. Dept. Agric. **ARS-33-42,** 24 pp.

Barthel, W. F., and B. H. Alexander. 1958. Preparation of the chrysanthemumates of 6-bromo- and 6-chloropiperonyl alcohols. Jour. Organ. Chem. **23**(7): 1012–4.

Chen, Yuh-Lin, and W. F. Barthel. 1956. Screening tests of esters of chrysanthemumic acid as synergists and insecticides. U. S. Dept. Agric. **ARS-33-23,** 10 pp.

Gersdorff, W. A. 1956. Effect of acetone on toxicity in pyrethrum and allethrin space sprays. Jour. Econ. Ent. **49**(6): 849.

Field Tests with Systemic Insecticides For the Control of Cattle Grubs[1]

W. W. NEEL,[2] *Mississippi Agricultural Experiment Station, State College*

ABSTRACT

Three systemic insecticides were compared during 1957–58 in Mississippi in an effort to establish the relative effectiveness of these materials for the control of the common cattle grub, *Hypoderma lineatum* (DeVill.). Dow ET-57 (Trolene), administered as a bolus, and Bayer 21/199 (Co-Ral), administered as a spray, when applied in the late summer or early autumn to test groups of Hereford cattle were equally effective in giving significantly better control of grubs than Am. Cyanamid 12880 (Dimethoate). The groups treated with dimethoate, administered as a bolus and as an intramuscular injection, were observed to have significantly fewer grubs than the groups which received no treatment.

No significant weight gain due to grub treatments could be shown. The failure to show weight gains may have been due to the fairly low grub population encountered during the period of these tests. The average numbers of grubs per untreated animal on four test herds were 13.1, 16.1, 11.0 and 11.9.

One herd of brood cows at Poplarville, Mississippi, was treated with phenothiazine for anthelmintic action at the same time the grub control materials were administered. Although no weight response attributed to the action of the systemic materials was shown in this herd, a significant weight gain due to the action of phenothiazine was demonstrated.

In the past 3 or 4 years considerable interest has been directed toward the success of several new systemic insecticides for the control of the two species of cattle grubs, *Hypoderma lineatum* (DeVill.) and *H. bovis* (L.). The promising results of Dow ET-57 (Trolene) given orally at the rate of 100 mg./kg. of body weight have been reported by McGregor & Bushland (1957) and Roth & Eddy (1957). Several workers including Adkins (1957), Howell (1957) and DeFoliart & Glenn (1957) have verified the effectiveness of the oral administration of Dow ET-57 for cattle grub control. Raun & Herrick (1957) have shown that the elimination of grubs from feed lot steers resulted in better gains in weight and in better feed conversion.

Other systemics that have been field tested in the past 3 years have been Bayer 21/199 (Co-Ral) and Am. Cyanamid 12880 (dimethoate) (*O,O,*-dimethyl S-(N-methylcarbamoylmethyl) phosphoro-dithioate. Brundrett *et al.* (1957) reported the effectiveness of Bayer 21/199 as a dermal spray when applied as an emulsion or suspension at a concentration of 0.75%. The tests of Am. Cyanamid 12880 in the field have not been as extensive as those of the other two materials because Am. Cyanamid 12880 has been released just recently. It has been tried most frequently at rates of 10 and 15 mg./kg. of body weight in the form of an intramuscular injection, as a bolus and as a drench.

The encouraging results with Dow ET-57 during the 1956–1957 grub season in South Mississippi (Neel 1957) pointed out the need for additional work with these new systemics. Accordingly, two other materials, Bayer 21/199 and Am. Cyanamid 12880, were tested in the field at three locations during 1957–1958.

PROCEDURE.—All animals in this series of tests were treated between August 20 and October 14, 1957. The October treatments might be considered a little late since the presence of grubs in the backs of animals can be detected in the southern part of the state during the latter part of October and the first week of November.

At each location the herd was divided into four groups and selections were made, wherever possible, so that

[1] Journal Publication No. 734 of the Mississippi Agricultural Experiment Station. Accepted for publication May 26, 1958.

[2] An expression of appreciation is due Messrs. E. G. Morrison, Superintendent, Brown Loam Branch Station, and Clyde L. Blount, Agronomist, South Mississippi Branch Station, for their cooperation in making these tests possible.

Table 1.—Grub control and weight response of Hereford heifers treated with systemic insecticides in Mississippi, 1957–1958.

Treatment	Observation Period (Days)	Total No. Animals per Group	Average No. Grubs per Animal	Average Weight in Pounds	
				Initial	Gain or Loss
Treated Aug. 20, 1957 at Raymond					
Dow ET-57	161	10	1.0	472	13.0
Bayer 21/199		10	2.4	482	16.0
Am. Cyanamid 12880		10	7.6	455	4.5
Control		9	13.1	516	9.4
L.S.D. at 5% level			2.86		n.s.
at 1% level			4.38		
Treated Oct. 14, 1957 at Raymond					
Dow ET-57	106	11	0.54	377	−20
Bayer 21/199		11	0.73	390	− 7
Am. Cyanamid 12880		11	4.91	386	−20
Control		11	16.09	374	−37
L.S.D. at 5% level			2.84		n.s.
at 1% level			4.31		
Treated Aug. 27, 1957 at Woodville					
Dow ET-57	154	12	2.63	655	0.8
Bayer 21/199		12	2.18	638	12.1
Am. Cyanamid 12880		12	6.36	628	−13.3
Control		12	11.00	662	7.1
L.S.D. at 5% level			2.84		n.s.
at 1% level			4.31		

equal numbers, according to age and weight classes, were represented in each group.

Dow ET-57 was administered in all the tests in the form of a bolus at the rate of 110 mg./kg. (approximately 5 gm./cwt.). Each bolus contained 15 grams of active material and was used to treat 300 pounds of live weight per animal.

Am. Cyanamid 12880 was administered at the rate of 10 mg./kg. (455 mg. of drug/100 lbs. body weight). It was administered as a bolus to all animals except the herd at Woodville, Mississippi, where it was given as an intramuscular injection (50% active ingredient).

Bayer 21/199 was made up from a 25% wettable powder and was applied externally as a 0.5% aqueous spray to the backs and sides of the treated animals. Hydraulic sprayers were used to apply this material at pressures of 200 p.s.i. or greater. Animals were thoroughly wetted with the insecticide. An average amount of 2 quarts was applied to each adult animal with the exception of young heifers which were treated with lesser amounts of the material.

In order to determine the influence of these systemic materials on weight responses, all the test animals were weighed with scales at the beginning and at the end of the observation period. Grub counts were taken at monthly intervals and grub infestations were charted by means of individual back diagrams so that cumulative totals could be obtained.

LOCATION OF TESTS.—*Raymond.*—This test was located at the Brown Loam Branch Station (Mississippi Agricultural Experiment Station). One group of Hereford heifers was treated on August 20, 1957; the other group of younger Hereford heifers, being weaned at the time, were treated on October 14, 1957. Identification of each animal was made by means of ear notches. Both groups had been on Bermuda-Dallis grass pasture during the summer and early fall months. Later during the fall and winter they were fed 25 pounds of silage and 1½ pounds of cottonseed meal per head per day.

Woodville.—All of the 48 Hereford cattle in this test were bred heifers belonging to the Kee Ranch herd. Treatments were made on August 27, 1957. Ear tags were used to identify the animals. Although a few grubs were noted in this herd during the latter part of October, the peak of the population did not appear until the first week in January. The pastures were chiefly Bermuda and Dallis grass during the summer and early fall months. Later the cattle were fed hay, free-choice, and cottonseed meal after the failure of winter pastures consisting of oats and rye grass.

Poplarville.—The cattle in this test were 48 Hereford brood cows belonging to the South Mississippi Branch Experiment Station. The herd was divided into four groups of 12 animals each. In order to determine the effectiveness of internal parasite control at the time of grub treatment, each of the groups was divided so that six animals received phenothiazine and six did not. Phenothiazine was administered as a drench at the recommended dose of 50 grams per head. Branding numbers were used to identify each animal. The animals were on Bahia and coastal Bermuda pasture at the time of treatment and were later fed silage and grass hay, free-choice, and 1 pound of cottonseed meal per day.

RESULTS AND DISCUSSION.—*Grub Control.*—In respect to grub control with the three materials a fair degree of uniformity prevailed throughout the state although there was a decided age-class difference in animals at the three locations. Dow ET-57 and Bayer 21/199 persistently gave better grub control than Am. Cyanamid 12880. No significant difference could be shown between the low grub counts resulting from the use of the first two materials. Although the performance of Am. Cyanamid 12880 at the dosage used was definitely poorer than the other two systemics the animals treated with this material had

Table 2.—Grub control and weight response of Hereford Brood cows treated simultaneously with systemic insecticides and phenothiazine at Poplarville, Mississippi, on September 25, 1957.

	No. Animals per Group	Average No. Grubs per Animal	Weight in Pounds	
			Average Initial	Total[a] Gain
Dow ET-57	6	0.5	980	0
Dow-57+phenothiazine	6	0.17	989	105
Bayer 21/199	6	0.17	987	110
Bayer 21/199+phenothiazine	6	0.0	903	220
Am. Cyanamid 12880	6	2.0	988	55
Am. Cyanamid 12880+phenothiazine	5	4.83	956	90
Control	6	9.17	923	55
Control+phenothiazine	6	14.67	895	105
Total—no phenothiazine			970	220
Total—phenothiazine			936	520[b]
Systemic treatments—				
L.S.D. at 5% level		2.84		n.s.
at 1% level		4.31		n.s.

[a] Period of observation was 79 days.
[b] Significantly different at the 5% level.

significantly fewer grubs than the animals which received no treatment. The number of grubs actually present in the different groups is shown in table 1.

Weight Response.—In regard to gain or loss in weight, significant differences due to treatments were not shown. Surprisingly enough, the gain in weight during the 3 to 5 month period following treatments was very small and in one group a reversal of the expected trend was noted. This group, consisting of 44 replacement heifers, was weaned at the time of treatment on October 10 and was noted to lose weight during the 106 day observation period.

A noteworthy weight response due to phenothiazine was noted at the Poplarville Station (table 2) when this material was administered as an anthelmintic agent at the time of the grub treatments. Although no significant gain in weight due to grub treatments was shown, a decided weight response due to the action of this anthelmintic was noted. The gain in weight of the phenothiazine-treated animals was significantly better than those which received no phenothiazine. The cattle showed no adverse effects following the simultaneous treatment with phenothiazine and the systemic insecticides.

REFERENCES CITED

Adkins, T. R. 1957. Field evaluation of Dow ET-57 as a systemic insecticide for the control of the common cattle grub in Alabama. Jour. Econ. Ent. **50**(4): 474–6.

Brundrett, H. M., W. S. McGregor, and R. C. Bushland. 1957. Systemic cattle grub control with Bayer 21/199 sprays. Agric. Chemicals 12(6): 36–37.

Defoliart, G. R., and M. W. Glenn. 1957. Cattle grub and lice control with Dow ET-57 in Wyoming. Down to Earth. 13(3): 4–5.

Howell, D. E. 1957. Grub control with Dow ET-57, a recently developed systemic insecticide. Feeders Day Rept, Oklahoma State University, 103–4.

McGregor, W. S., and R. C. Bushland. 1957. Tests with Dow ET-57 against two species of cattle grubs. Jour. Econ. Ent. **50**(3): 246–9.

Neel, W. W. 1957. New drug given internally to control cattle grubs. Mississippi Farm Res. 20(6): 8.

Raun, E. S., and J. B. Herrick. 1957. Clinical test of the efficacy of Dow ET-57 for grub control in cattle. Jour. Econ. Ent. **50**(6): 832.

Roth, A. B., and Gaines W. Eddy. 1957. Tests with Dow ET-57 against cattle grubs in Oregon. Jour. Econ. Ent. **50**(3): 244–6.

Horn Fly Control by the Use of Insecticidal Dusts in Self-Applicating Devices[1]

L. T. HARGETT,[2] and E. C. TURNER, JR., *Virginia Agricultural Experiment Station, Blacksburg*

ABSTRACT

Toxaphene, chlordane and Bayer 21/199 dusts were used in cable-type backrubbers for horn fly control on beef cattle. Methoxychlor and Bayer 21/199 dusts were also used in a special cable-type backrubber for horn fly control on dairy cattle. Backrubbers treated with insecticides diluted in oil were used as a standard. The data obtained demonstrated that these materials in a dust form may be used successfully in self-applicating devices for the control of horn flies on beef and dairy cattle.

In recent years rapid progress has been made in control of the horn fly, *Siphona irritans* (L.), by the use of self-applicating devices. Rogoff (1952), Rogoff & Moxon (1952), and Lindquist & Hoffman (1954) all obtained excellent horn fly control of range cattle by the use of cable-type backrubbers treated with oil solutions of chlorinated hydrocarbon insecticides. Since this and subsequent research dealt with backrubbers treated with liquid-base insecticides, it was felt that backrubbers treated with dust-base insecticides might offer a supplemental method of controlling horn flies.

Preliminary data obtained from experiments designed and executed during the late summer of 1956 indicated that horn fly control could be obtained on beef cattle by the use of backrubbers treated with insecticides diluted with dust. Further experiments were conducted during the summer of 1957 to verify these results, and also to determine if a similar method would be feasible for dairy cattle.

DUSTS IN CABLE-TYPE BACKRUBBERS.—A conventional triangle frame was installed for the dust-type backrubber for beef cattle. Before the burlap sacks were wrapped around the barbed wire, three-fourths of a pound of dust-diluted insecticide was poured into each. Half of each sack was then wrapped around the barbed wire core and one-fourth of a pound of additional insecticide was poured into the crease formed between the wrapped portion of the sacks and the unwrapped portion. The sacks were then completely wrapped around the barbed wire and tied in the usual manner. Approximately 1 pound of dust was used to each sack required, or 14 pounds to each backrubber. The sacks used were small mesh burlap feed sacks.

A backrubber constructed in the manner just described and treated with 10% toxaphene dust was installed in each of three pastures. These pastures were very similar in size, terrain and number of Hereford cattle in each. All backrubbers were treated and installed the same day. Pre-treatment counts of horn flies were taken, after which post-treatment counts were made weekly. The three backrubbers gave good control of horn flies on these herds for a period of 3 weeks, at which time it was necessary to terminate the experiment.

The results of this preliminary experiment are given in table 1. These results were so favorable that further experiments of this nature were conducted during the summer of 1957 on both beef and dairy cattle.

An experiment was designed to determine how long backrubbers treated with certain dust-diluted insecti-

[1] Part of a thesis by the senior author submitted to the faculty of Virginia Polytechnic Institute in partial fulfillment of the requirements for the Degree of Master of Science in Entomology. Presented at the Fifth Annual Meetings of the Entomological Society of America, Memphis, Tennessee, December 3, 1957. Accepted for publication May 26, 1958.

[2] Present address: Department of Entomology, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Table 1.—Effectiveness of backrubbers treated with 10% toxaphene dust. Giles County, Virginia. 1956.

Herd No.	Average No. of Horn Flies per Animal			
	Before Treatment	After Treatment (Weeks)		
		1st	2nd	3rd
1	268	3.8	11.6	4.6
2	233	1.0	35.7	3.8
3	238	.1	16.7	3.6

cides would give satisfactory control and also to determine if the control obtained would be comparable to that obtained from backrubbers treated with oil-diluted insecticides. It was also desirable to determine what effect rain would have on the control from backrubbers treated with dust.

Twelve backrubbers were constructed in as many pastures, all of which were treated the same day. Three were treated with 5% toxaphene dust, three with 5% toxaphene oil, three with 5% chlordane dust and three with 5% chlordane oil. One of each type application was treated every month, one every 2 months, and one received only the initial treatment. Horn fly counts were made weekly on the treated herds and also on the untreated check herds.

Two backrubbers were constructed in pastures of approximately the same size and terrain; one was treated with 1% Bayer 21/199 dust and the other was treated with 2% Bayer 21/199 dust. Counts were made weekly until the control ceased.

Table 2 shows that toxaphene dust gave satisfactory control of horn flies (that is, at each observation the herds averaged 60 or less flies per animal) for 7.3 weeks while the toxaphene oil gave 5.7 weeks of control. Chlordane dust and chlordane oil each gave 16 weeks of effective control. One per cent Bayer 21/199 dust gave satisfactory control for 5 weeks, however, 2% Bayer 21/199 dust gave this control for only 3 weeks. The probable reason 2% Bayer 21/199 gave a shorter period of control than the 1% is that the cooperator opened additional pastures while the experiment was in progress.

From 1, 2, and 4 treatments, respectively, satisfactory fly control was obtained from the designated materials for the period indicated: toxaphene, 5% dust, 1, 10, and 13 weeks; toxaphene, 5% oil solution, 4, 3, and 14 weeks; chlordane, 5% dust, 13, 14, and 14 weeks; and chlordane, 5% oil solution, 13, 1, and 15 weeks. Based on a total of 45 observations each formulation of insecticide gave a definite number of weeks of good horn fly control (that is, an average of 20 or less flies an animal per observation). Toxaphene dust and toxaphene oil gave good control for 24 and 21 weekly observations, respectively. Chlordane dust and chlordane oil gave this control for 41 and 29 weekly observations, respectively. The reason for the considerable difference between chlordane dust and chlordane oil is probably due to a high initial horn fly population on the herd that was treated twice with chlordane oil. A considerable reduction in the horn fly population was observed on this herd, but not enough to cause it to fall into the above grouping for more than one time.

Table 2.—Longevity of effectiveness of backrubbers treated with different materials. Montgomery County, Virginia. 1957.

Material	Diluent	Weeks of Effective Control[a]	Replicates
5% toxaphene	dust	7.3	3
5% toxaphene	oil	5.7	3
5% chlordane	dust	16.0	1
5% chlordane	oil	16.0	1
1% Bayer 21/199	dust	5.0	1
2% Bayer 21/199	dust	3.0	2

[a] Based upon an average population of 60 or less flies per animal.

Rainfall, as would be expected, had some effect on the control given by the backrubbers treated with insecticides diluted with dust. The graph clearly illustrates that the rainfall did not have a greater effect on the dust-diluted insecticides than it did on the oil-diluted insecticides (fig. 1.). The toxaphene dust and oil was influenced to a greater extent by the rainfall than the chlordane dust and oil. The rainfall had very little effect on the control given by the chlordane treatments.

Dairy Backrubbers.—The need for an easy, economical method for controlling horn flies on dairy cattle has been apparent for some time. The backrubber offered what seemed to be the best approach to this problem. The conventional type backrubber was not practical because it had to be relocated each time the pasture was rotated by the dairyman. The following modification was used. A heavy chain was hung across the cattle walk leading into the dairy barn. Each end of the chain was fastened to a screw hook about 4½ feet from the ground. The center of the chain was allowed to hang about 32 inches above the ground (fig. 2.)

When treated with an insecticide diluted with fuel oil the sacks were folded into quarters, placed end to end, wrapped around the chain and tied securely. The backrubber was then saturated with the insecticide in the conventional manner. When dust was used as the diluent, the following procedure was followed: A pound of the prepared insecticide was placed in the bottom of each sack to be used. The sacks were then placed end to end and wrapped around the chain and tied at each end and in the center. The cattle walked under the backrubbers twice a day, once after each milking. The result was that a liberal application of insecticide was applied to the back and upper side of the cattle as they passed under it.

Two backrubbers were treated with 10% methoxychlor dust, two with 5% methoxychlor oil, two with 1% Bayer 21/199 dust and one with 25% methoxychlor dust. Weekly observations were made on 10 Holstein cows in each herd which had access to a dairy backrubber and also on 10 animals in an untreated herd. All experiments except the 25% methoxychlor dust were replicated four times. The average number of weeks of satisfactory control, that is, an average of 50 or less flies per animal, is given in table 3.

The results obtained from these experiments indicate

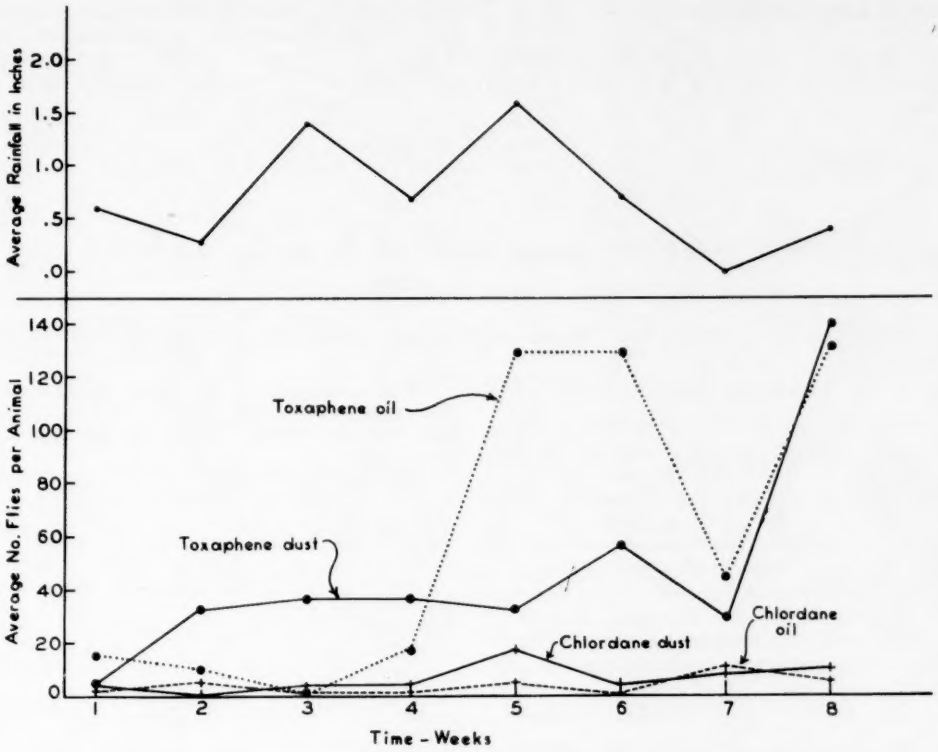


FIG. 1.—The effect of rainfall on the control of horn flies obtained by the use of backrubbers.

that backrubbers treated with dust-diluted insecticides result in as good control of horn flies on beef cattle as that of backrubbers treated with oil-diluted insecticides. This brings up the question regarding possible residues of toxaphene and chlordane in meat of animals using these dust-type backrubbers. No tolerance has been set for chlordane and only toxaphene, methoxychlor, and DDT may be recommended in oil-type backrubbers on beef cattle.

A more serious question is whether the use of the above-mentioned insecticides, applied in the dust-type backrubber, would result in residues in milk. At the present time, only methoxychlor, applied as a dust to the backs of animals, is recommended for use on lactating dairy cows. Further research is needed to compare the amount of insecticide residue left in milk and meat of animals treated with both oil- and dust-diluted insecticides.

Table 3.—Average number of weeks of horn fly control given by dairy backrubbers. Montgomery County, Virginia. 1957.

INSECTICIDE	DILUENT	REPLICATES	WEEKS OF CONTROL[a]
25% methoxychlor	dust	1	8.0
10% methoxychlor	dust	4	5.5
5% methoxychlor	oil	4	4.5
1% Bayer 21/199	dust	4	3.5

[a] Based upon 50 or less flies per animal.

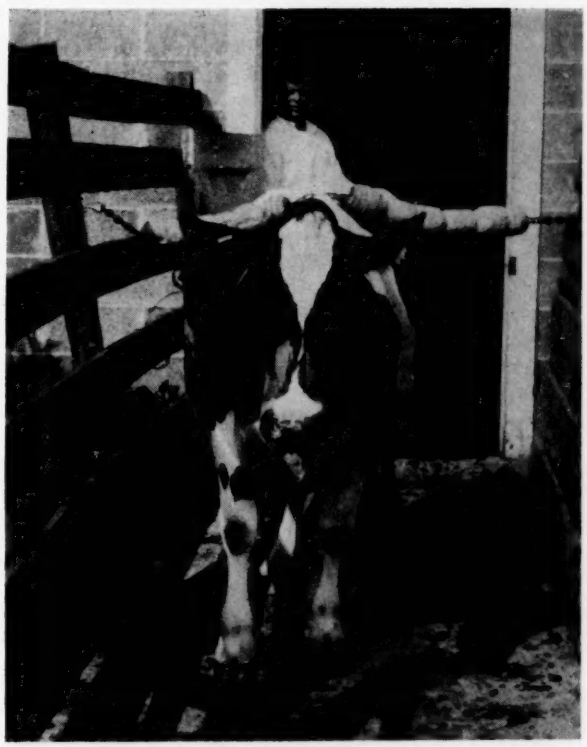


FIG. 2.—Completed dairy backrubber.

REFERENCES CITED

Lindquist, A. W., and R. H. Hoffman. 1954. Effectiveness of cattle rubbing devices and hand dusting for horn fly control. Jour. Econ. Ent. **47**: 79–81.

Rogoff, W. M. 1952. Cable-type backrubbers for horn fly control on cattle. South Dakota Bull. **418**, 12 pp.

Rogoff, W. M., and A. L. Moxon. 1952. Cable-type backrubbers for horn fly control on cattle. Jour. Econ. Ent. **45**: 329–34.

Synergized Pyrethrins and Allethrin Baits for the Control of Resistant House Flies[1]

G. C. LABRECQUE, H. G. WILSON, and J. B. GAHAN,[2] *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

ABSTRACT

Pyrethrins and allethrin synergized by piperonyl butoxide at a ratio of 1:10 and incorporated in sugar baits were effective against organophosphorus-resistant house flies (*Musca domestica* L.) in laboratory tests and field tests in dairy barns of central Florida. In laboratory tests both were less effective than Dipterex against susceptible flies, but pyrethrins were about equal against a malathion-resistant strain. In field tests pyrethrins at 0.1% were superior to allethrin at 0.1%, which in turn was more effective than Dipterex at 1% in the reductions obtained 10 minutes after treatment. In the 24-hour reductions pyrethrins were again superior to the allethrin, which was equal to Dipterex.

Organophosphorus insecticides, particularly malathion and Dipterex, incorporated in dry granular baits have been utilized extensively to control house flies (*Musca domestica* L.) in and around poultry houses and dairy barns in Florida. Within the last 2 years flies on some premises became so resistant to these insecticides (LaBrecque *et al.* 1957, 1958) that it became evident that other insecticides were necessary to re-establish fly control.

In the spring of 1957 investigations were undertaken to evaluate various groups of compounds as toxicants in baits. The materials were screened against both regular (susceptible) and malathion-resistant (Grothe) colonies of house flies. Pyrethrins and allethrin synergized with piperonyl butoxide at a 1:10 ratio were the most promising. Laboratory and field tests with these two insecticides and a Dipterex standard are reported in this paper.

LABORATORY TESTS.—Acetone solutions of the toxicants were mixed with dry sugar to form baits containing various concentrations of the insecticide. After the mixtures had dried overnight, 10 grams of each bait was placed in a half-section of a petri dish, and a small cylindrical cage containing 20 female flies was placed on the bait. The flies were allowed to feed ad libitum, the knockdown being recorded at the end of 1, 2, and 4 hours and the mortality after 24 hours. At the end of 4 hours a piece of absorbent cotton moistened with water was placed on the cage. Each concentration was tested at least twice against both colonies of flies. The results are summarized in table 1.

Pyrethrins and allethrin baits were not as effective as Dipterex at equal concentrations against the regular strain of house flies, but against the malathion-resistant strain pyrethrins were superior to Dipterex in knockdown and equal at 24-hour mortality counts. The Dipterex

Table 1.—Results of laboratory tests comparing the toxicity of baits containing Dipterex and synergized pyrethrins and allethrin to malathion-resistant and regular colony house flies.

INSECTICIDE (PER CENT)	PER CENT KNOCKDOWN IN—			PER CENT KILL IN 24 HOURS
	1 Hour	2 Hours	3 Hours	
Malathion-resistant colony				
Dipterex				
0.01	0	0	0	0
.025	0	0	0	10
.05	0	0	3	85
.1	0	0	13	98
.2	0	5	20	98
Pyrethrins				
0.01	3	3	8	23
.05	25	30	48	68
.1	25	28	35	53
.25	63	53	58	78
.5	75	90	75	90
1.0	45	55	58	90
Allethrin				
0.01	0	0	3	3
.05	15	15	20	33
.1	5	8	23	43
.25	58	63	63	60
.5	53	53	55	53
1.0	55	55	73	85
Regular colony				
Dipterex				
0.0005	0	0	0	0
.001	0	0	0	5
.0025	0	0	0	32
.005	0	0	0	98
.01	0	0	10	100
Pyrethrins				
0.01	3	5	3	28
.05	33	45	63	85
.1	43	58	65	85
.25	68	85	78	85
.5	50	60	65	80
1.0	48	56	70	85
Allethrin				
0.01	3	3	3	5
.05	3	3	3	33
.1	8	10	13	53
.25	23	25	33	53
.5	28	25	33	58
1.0	18	25	35	50

[1] Accepted for publication May 26, 1958.

[2] P. H. Adcock and C. R. Crittenden performed many of the routine duties connected with this research.

Table 2.—Effectiveness of baits containing synergized pyrethrins, synergized allethrin, or Dipterex when applied once against house flies in dairy barns.

Insecticide (Per Cent)	Pretreatment Count	Per Cent Reduction in—	
		10 Minutes	24 Hours
Dipterex			
1.0	135	37	80
	195	26	43
Pyrethrins			
0.05	135	98	93
	107	98	88
.1	129	97	91
	70	95	93
	85	99	87
	250	96	65
.25	142	97	95
	38	97	79
.5	101	98	65
	102	96	94
1.0	61	99	99
	83	96	95
Allethrin			
0.05	130	99	80
	20	98	22
.1	87	95	61
	250	97	20
	200	95	51
	60	98	83
.25	155	97	71
	143	96	94
	66	82	58
.5	112	97	99
	69	97	61
1.0	83	89	86
	172	98	97

baits were less effective against the malathion-resistant strain, whereas pyrethrins and allethrin were equally effective against both strains.

Field Tests.—Tests were run in unscreened dairy barns infested with flies, where previous tests had indicated a low to moderate resistance to organophosphorus baits. The baits were formulated in the same manner as in the laboratory tests. They were sifted lightly from a shaker-top jar on floors and entrances of feed and milking rooms where the flies congregated. From 100 to 200 grams were used per building, depending upon the area treated.

In one series of tests treatments were applied only once, but in a second series they were applied daily, Monday through Friday, for 3 weeks. The infestation was estimated by determining the number of flies that landed and fed in 2 minutes on cloth strips (1×24 inches) saturated with a 50% malt solution. Counts were made each day before the baits were applied and again after 10 minutes and 24 hours. At no time were any of the breeding areas on the premises treated.

The results of tests in which the baits were applied only once are given in table 2. Baits containing 0.05% of pyrethrins or allethrin were superior to Dipterex at 1% in causing a 10-minute reduction. Reductions after 24 hours were more variable, but concentrations as low as 0.05% of pyrethrins and 0.25% of allethrin were equal to or more effective than Dipterex at 1%.

Table 3.—Per cent reduction of house flies in dairy barns with sugar bait containing synergized pyethrins (0.1%) applied 5 days per week.

Days After First Application	Lakemont	Stevens	Judge	Brockbank	Palm	Hiatt
1	52	—	93	95	—	77
2	88	93	91	96	97	67
3	96	89	89	93	98	87
4	97	97	93	93	99	93
7[a]	85	74	0	63	95	21
8	75	88	87	31	—[b]	64
9	90	98	83	73	—[b]	22
10	88	89	83	90	72	58
11	98	80	88	88	97	90
14[a]	59	0	45	0	26	4
15	90	79	93	96	72	4
16	77	93	95	90	94	0
17	95	95	81	85	95	0
18	95	94	97	96	97	17
21[a]	46	82	38	57	91	45

[a] Indicated reduction after no treatment for 72 hours.
[b] No treatments applied because of low fly populations.

In the tests with daily applications for 3 weeks the pretreatment count before the first application ranged from 37 to 163 flies per cloth strip. Of the 88 applications with pyrethrins at 0.1%, 85 gave more than 80% reduction in 10 minutes, based on each day's pretreatment count. In two dairies the 10-minute reductions were greater than 92% throughout the entire test period. The 24-hour reductions, based on the count before the first application, were high in all but one of the dairies (table 3). In the Hiatt dairy, where control was poor, most of the breeding was in a neighboring calf-rearing area, which was exceptionally large and heavily infested.

Allethrin at 0.1%, although not so effective as pyrethrins, gave more than 80% reduction in 10 minutes in 49 out of 58 bait applications in four dairies. The 24-hour reductions were much less than with pyrethrins and about

Table 4.—Per cent reduction of house flies in dairy barns with sugar bait containing synergized allethrin (0.1%) or Dipterex (1%) applied 5 days per week.

Days After First Application	Allethrin 0.1% Plus Piperonyl Butoxide 1%				Dipterex 1%		
	Stevens	Barrows	Brockbank	Palm	Judge	Lakemont	Hiatt
1	—	38	85	—	77	91	87
2	—	49	93	—	94	95	91
3	84	38	94	90	94	90	90
4	94	89	97	93	94	95	90
7[a]	0	65	0	62	46	46	77
8	85	89	0	9	77	0	62
9	76	70	0	90	73	45	31
10	69	87	63	93	90	53	57
11	86	84	89	19	90	32	56
14[a]	55	73	41	84	17	0	0
15	97	60	92	90	87	54	0
16	98	68	94	88	79	21	75
17	94	62	83	49	67	65	61
18	92	30	93	90	58	80	82

[a] Indicated reduction after no treatment for 72 hours.

equal to those obtained with Dipterex at 1% (table 4). Dipterex baits were not highly effective in 10 minutes, reducing fly populations more than 80% only four times in 45 applications. Control with Dipterex was much less than that obtained in former years.

Throughout the tests bait applications were suspended over Saturday and Sunday. On only four occasions with pyrethrins and one with allethrin was the control above 80% the following Monday.

Results of these tests indicate that pyrethrins baits may reestablish good control in dairies where the flies have become resistant to organophosphorus insecticides. Thorough application at daily intervals to dairy barns and in the vicinity of breeding areas is required.

REFERENCES CITED

LaBrecque, G. C., and H. G. Wilson. 1957. House fly resistance to organophosphorus compounds in Florida. Agric. Chemicals **12**(9): 46–7, 147, 149.

LaBrecque, G. C., H. G. Wilson, and J. B. Gahan. 1958. Resistance of house flies in Florida to organophosphorus insecticides. Jour. Econ. Ent. **51**(5): 616–7.

Hydrolysis of the Alkyl-Phosphate Bond in Certain Dialkyl Aryl Phosphorothioate Insecticides by Rats, Cockroaches, and Alkali[1]

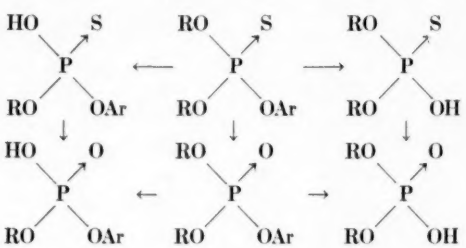
F. W. PLAPP[2] and J. E. CASIDA,[3] *Department of Entomology, University of Wisconsin, Madison*

ABSTRACT

The nature of the products formed by *in vitro* and *in vivo* hydrolysis of parathion, methyl parathion, Diazinon, Dow ET-57, Chlorthion, and dicapthon was studied. All these dialkyl aryl phosphorothioates were hydrolyzed at both the alkyl-phosphate and the aryl-phosphate bonds. Alkyl-phosphate hydrolysis was proportionately greater with the dimethyl than with the diethyl phosphorothioates in rats and under the alkaline conditions employed. In rats it was found that the per cent of hydrolytic metabolites formed by cleavage of the alkyl-phosphate bond varied with the amount of insecticide administered, being greater at higher dosages. Alkyl-phosphate hydrolysis appeared to be an alternate mechanism for detoxication in mammals when the dosage was too great to be metabolized through hydrolysis at the aryl-phosphate bond. It did not seem to be a major factor in the large toxicity differences among dialkyl aryl phosphorothioates with mammals.

Very little alkyl-phosphate hydrolysis occurred with cockroaches with five of the six compounds studied. The lower alkyl-phosphate hydrolysis with cockroaches as compared to rats may contribute to the lower relative toxicity of the dimethyl aryl phosphorothioates to mammals. Differences were also noted in the rate of oxidation of the various hydrolytic metabolites between rats and cockroaches.

The dialkyl aryl phosphorothioates form one of the most important groups of phosphate insecticides. Studies on the hydrolytic stability and anticholinesterase activity of compounds of this type have been based on the assumption that hydrolysis occurs only at the aryl-phosphate (acid anhydride) bond (Aldridge 1954, Fukuto & Metcalf 1956). However, it has been shown that Dow ET-57, (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) and its oxygen analog are hydrolyzed *in vitro* and *in vivo* at both the alkyl-phosphate and aryl-phosphate bonds (Plapp & Casida 1958b). This hydrolytic cleavage, combined with oxidation of the phosphorothioate grouping either before or after hydrolysis, yielded four nontoxic, water-soluble phospho-diesters. The metabolism of dialkyl aryl phosphorothioates can therefore be represented as follows:



The significance of alkyl-phosphate hydrolysis as a detoxication mechanism and as a factor in selective toxicity was investigated further with parathion, methyl parathion, Diazinon, Dow ET-57, Chlorthion, and dicapthon.

METHODS AND RESULTS.—*Alkaline Hydrolysis.*—The stability to hydrolysis by alkali and the nature of the products formed was studied using previously described techniques (Plapp & Casida 1958a, 1958b). The insecticides used were of the highest purity available from the manufacturer[4] and were further purified by dissolving in chloroform and extracting with 10% aqueous sodium carbonate and then with water. The per cent hydrolysis under alkaline conditions was determined by incubating the purified organophosphates with equimolar potassium hydroxide in 95% ethanol at a concentration for each of 25 micromoles per ml. The nature of the products formed was determined using ion exchange and paper chromatography. These hydrolysis conditions were selected since they were found to yield the highest proportion of mono-

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and by a grant from the U. S. Atomic Energy Commission (Contract No. AT(11-1)-64, Project No. 14). Accepted for publication May 28, 1958.

[2] Present address: Entomology Research Division, Agricultural Research Service, U.S.D.A., Corvallis, Oregon.

[3] The authors wish to acknowledge the skilled technical assistance of Miss Anda Dimze, Mr. T. K. Knoke, and Mrs. Sally Jo Krueger.

[4] The insecticides used in this study were obtained as follows: parathion, methyl parathion, and dicapthon from the American Cyanamid Co.; para-oxon from the Victor Chemical Works; Chlorthion from Farbenfabriken Bayer: Dow ET-57 and analogs from The Dow Chemical Co.; and Diazinon from the Geigy Chemical Corp.

Table 1.—Compounds studied and alkaline hydrolysis results.

COMPOUND	FORMULA	PER CENT HYDROLYSIS BY EQUIMOLAR KOH IN 95% ETHANOL AFTER 20 HOURS	PER CENT HYDROLYSIS PRODUCTS AS RO(ØO)P-(S OR O)OH[a]	R_f VALUES FOR RO(ØO)P(S)OH[b]
Dow ET-57	$(CH_3O)_2P(S)OØ$-2,4,5Cl_3	46.2±1.8[c]	64	0.91±0.02[c]
	$(CH_3O)_2P(O)OØ$-2,4,5Cl_3	68.8±1.4	49	0.79±0.01[d]
	$(C_2H_5O)_2P(O)OØ$-2,4,5Cl_3	38.3±0.7	29	—
Dicapthon	$(CH_3O)_2P(S)OØ$-2Cl,4NO_2	34.5±1.9	46	0.78±0.03
Chlorthion	$(CH_3O)_2P(S)OØ$-3Cl,4NO_2	34.6±1.1	35	0.83±0.01
Methyl parathion	$(CH_3O)_2P(S)OØ$-4NO_2	32.6±1.3	46	0.74±0.02
Parathion	$(C_2H_5O)_2P(S)OØ$-4NO_2	9.0±1.1	11	—
Para-oxon	$(C_2H_5O)_2P(O)OØ$-4NO_2	40.7±1.1	12	—
Diazinon	$(C_2H_5O)_2P(S)O$-(2-isopropyl-4-methyl-6-pyrimidinyl	12.7±1.0	9	—

[a] Figures are average of two replicates. Remaining products are dialkyl phosphoric or phosphorothioic acids.
[b] Single phase ascending paper chromatography. Developing solvent 3 parts 99% isopropanol; 1 part concentrated ammonium hydroxide. R_fs of $(MeO)_2P(S)OH$, 0.65±0.02; $(EtO)_2P(S)OH$, 0.77±0.02; $(MeO)_2P(O)OH$, 0.44±0.01; $(EtO)_2P(O)OH$, 0.61±0.01 (Plapp & Casida 1958a).
[c] Standard deviation.
[d] As potassium salt (Plapp & Casida 1958a).

alkyl aryl phosphoric and phosphorothioic acids. The primary hydrolysis products formed, diesters of phosphoric and phosphorothioic acid, are stable under these conditions (Kosolapoff 1950) and in no cases were hydrolysis products other than diesters recovered.

Compounds included in the study, hydrolysis data, and R_f values obtained for certain of the products are presented in table 1. The dimethyl compounds were hydrolyzed more readily than the diethyl compounds and the phosphates more readily than the phosphorothioates. A higher percentage of hydrolysis at the alkyl-phosphate bond occurred with the dimethyl than with the diethyl derivatives. Where the aryl group was 2,4,5-trichlorophenol, the proportion of aryl-phosphate hydrolysis was lower than with the other aryl groups included in the study.

Synthesis and Characterization of Labeled Compounds.— The six phosphorothioate insecticides listed in table 2 were synthesized from phosphorus-32 pentasulfide prepared by isotope exchange (Casida 1958). The phosphorus pentasulfide was converted to dialkyl phosphorodithioic acid and dialkyl chlorophosphorothioate (Fletcher *et al.* 1950) and finally to the *O,O*-dialkyl *O*-aryl phosphorothioates (Fletcher *et al.* 1950, Louloudes *et al.* 1956). The specific activity of the compounds studied ranged from 2 to 5 millicuries per gram.

The compounds were purified by partition chromatography on columns (Bowman & Casida 1957) and the specific activity of the products found to be constant throughout the elution peaks. All radioactive compounds were identical in infrared spectra (10% solutions in chloroform, Baird spectrophotometer, sodium chloride optics) and in toxicity to house flies (Krueger & Casida 1957) to the known phosphorothioates.

Hydrolysis of the radioactive insecticides in 50% ethanol with strong potassium hydroxide yielded 90% or more dialkyl phosphorothioic acids based on a paper chromatographic system which separated the potassium

Table 2.—Hydrolysis products from rats and cockroaches after treatment with phosphorus-32 labeled insecticides.[a]

COMPOUND	DOSE, μGM./GM.	PER CENT HYDROLYSIS PRODUCTS AS $(RO)_2P(O)OH$	$(RO)_2P(S)OH$	$RO(ØO)P(O)OH$	$RO(ØO)P(S)OH$
		Rats			
Dow ET-57	2	61	24	9	6
	100	15	18	25	42
Dicapthon	100	37	26	10	27
Chlorthion	100	29	33	13	25
Methyl parathion	2	60	27	4	9
Parathion	1.5	16	78	2	4
Diazinon	20	30	65	—	5[b]
	100	24	71	—	5[b]
		Cockroaches			
Dow ET-57	20	16	76	1	7
Dicapthon	20	3	93	—	4[b]
Chlorthion	20	2	97	—	1[b]
Methyl parathion	4	27	47	3	23
Parathion	20	28	67	3	2
Diazinon	20	41	59[c]	—	—

[a] Figures from rats are average of two replicates. Cockroach results are from one replicate only.
[b] Includes trace amounts of the oxygen analog of this compound.
[c] Includes trace amounts of monoalkyl aryl phosphate and phosphorothioate.

O,O-dialkyl phosphorothioates from the potassium *O*-alkyl *O*-aryl phosphorothioates. The technique employed was single phase ascending paper chromatography on Whatman #1 paper with acetonitrile and water, 85:15, as the developing solvent (Robbins 1957). This indicated that little if any *O,O*-diaryl *O*-alkyl phosphorothioate impurities were present in the radioactive preparations. The described synthesis route via phosphorus pentasulfide and the dialkyl phosphorodithioic acid is much less likely to yield large amounts of diaryl impurities than the route using thiophosphoryl trichloride to prepare the dialkyl chlorophosphorothioate.

The purity of the radioactive compounds was also determined with alumina columns. The columns were prepared as follows: 10 grams of aluminum oxide, (Woelm, acid (anionotropic), activity grade I for chromatographic analysis) and 0.2 ml. of water were mixed, and the hydrated aluminum oxide slurried in hexane and poured in a column one cm. in diameter to a depth of 14 cm. A hexane solution of the compound to be chromatographed (not more than 2 μgm.) was then pipetted onto the column. One hundred and fifty ml. of each of the following solvents were run through the column: hexane, benzene, chloroform, and methanol. The phosphorothioates shown in table 1 were eluted entirely in the benzene fraction and the phosphates in chloroform. All the radioactive phosphorothioates were 98 to 99.5% pure based on this procedure.

In vivo Metabolism Studies.—Rats.—The phosphorus-32 labeled insecticides were dissolved in a mixture of 1 part acetone and 4 parts corn oil and administered orally to 200-gram white rats. Two male and two female rats were treated with each compound at approximately 0.05 to 0.10 of the LD_{50} dose (table 2). No more than mild transient symptoms of phosphate poisoning were observed. When large doses were required (100 mg. per kg.), non-radioactive carrier insecticide was mixed with the radioactive sample. Dow ET-57 was also administered to rats at the same level as the more toxic methyl parathion (2 mg. per kg.), and Diazinon was administered at the same level as the less toxic dimethyl compounds (100 mg. per kg.).

The two animals of each sex treated with each compound were held in separate metabolism cages (Comar 1955) to determine metabolism differences between the sexes. Large differences exist in the toxicity of certain organophosphates to male and female rats, the females usually being more susceptible (Frawley *et al.* 1952).

Urine samples from the treated rats were collected, assayed for total radioactivity, and held at 4° C. until analyzed by ion exchange chromatography. The largest proportion of the total radioactivity excreted was recovered in the first 24 hours after treatment (69 to 97%), with Diazinon being excreted the fastest and Dow ET-57 and dicapthon the slowest. Greater than 99% of the recovered radioactivity was in the form of water-soluble hydrolysis products based on extraction with chloroform.

The nature of the hydrolytic metabolites from the administered compounds was determined by the ion exchange technique previously described (Plapp & Casida 1958a). The identity of the excreted radioactive metabolites was verified by chromatography with carrier compounds prepared as follows: hydrolysis of the administered insecticide in alcoholic potassium hydroxide yielded mixtures of the *O,O*-dialkyl and *O*-alkyl *O*-aryl phosphorothioic acids, and hydrolysis of dialkyl phosphates (TEPP and Phosdrin) in water with strong alkali yielded the *O,O*-dialkyl phosphoric acids. The *O*-alkyl *O*-aryl phosphoric acids were the only metabolites not chromatographed with carrier compounds. The site of elution of compounds of this type has been studied (Plapp & Casida 1958a), and in all cases the metabolites of the compounds included in the study were eluted from the columns in the same order and with the same solvents as previously reported.

The results of the rat metabolism studies are presented in table 2. No consistent differences between male and female rats were noted and the figures given represent average values for the metabolites from both sexes. Hydrolysis of all compounds studied occurred at both the alkyl-phosphate and the aryl-phosphate bonds. With the diethyl phosphorothioates the proportion of alkyl-phosphate hydrolysis was very small and as shown with Diazinon was not found to vary with the amount of compound administered. Alkyl-phosphate hydrolysis was proportionately greater with the dimethyl phosphorothioates and varied with the level of treatment and with the different aryl derivatives. With Dow ET-57 the proportion of alkyl-phosphate hydrolysis was much greater at 100 mg. per kg. than at 2 mg. per kg. The proportion of alkyl-phosphate hydrolysis was greater with Dow ET-57 than with dicapthon or Chlorthion at 100 mg. per kg., but was not greatly different with Dow ET-57 and methyl parathion at 2 mg. per kg.

Differences in the rate of oxidation of the phosphorothioate grouping were noted with the diethyl and the dimethyl phosphorothioates. The proportion of dialkyl and monoalkyl monoaryl phosphoric acids was larger with the dimethyl than with the diethyl derivatives. The proportion of oxidized derivatives varied with the level of treatment, being greater at the lower levels.

Cockroaches.—Groups of 25 to 50 adult male and female American cockroaches, *Periplaneta americana* (L.), were treated with the radioactive phosphorothioates by topical application on the pronotum in 1.3 μl. acetone at levels indicated in table 2. The treated insects were held at room temperature for 20 hours and then macerated in a Waring blender, first in acetone and then in benzene. One hundred ml. of each solvent were used per 25 grams of cockroaches. The solvents were separated from the residue by filtration on a Buchner funnel, pooled and evaporated to dryness on a steam bath and then partitioned with 50 ml. each of n-hexane and water. Using this method it was found that all the insecticides went greater than 99% into hexane, the hydrolysis products greater than 99% into water.

From 11 to 78% of the extracted radioactivity was water-soluble and considered to be hydrolysis products. The proportion was highest (78%) with methyl parathion at 4 μgm. per gm., intermediate (27 to 36%) with the diethyl phosphorothioates, and lowest (11 to 12%) with the dimethyl phosphorothioates administered at 20 μgm. per gm. Using alumina columns as previously described, evidence was obtained for the partial conversion *in vivo* of all the phosphorothioates to the corresponding phosphates.

The water-soluble radioactivity was chromatographed on ion exchange columns (table 2). With the exception of methyl parathion, the proportion of alkyl-phosphate hydrolysis was much lower than in the rat experiments. A larger proportion of alkyl-phosphate hydrolysis occurred with methyl parathion administered at 4 μgm. per gm. than with the other dimethyl and diethyl phosphorothioates, all of which were administered at 20 μgm. per gm. of cockroach. The proportion of dialkyl phosphoric acid formed in relation to dialkyl phosphorothioic acid was greater with the diethyl than with the dimethyl derivatives.

DISCUSSION.—The higher percentage of hydrolysis at the alkyl-phosphate bond by rats as compared with cockroaches, particularly with the dimethyl phosphorothioates, suggests that this method of detoxication may be more important with mammals than with insects. Similar results have been reported for Dow ET-57 with rats and house flies (Plapp & Casida 1958b).

The site of hydrolytic attack by rats on the dimethyl compounds was greatly affected by the level of administration. Comparable results were obtained in previous experiments where it was demonstrated that after oral administration of Dow ET-57 or its oxygen analog to rats, the per cent of alkyl-phosphate hydrolysis decreased with time, *e.g.*, as the level of insecticide in the animal body declined (Plapp & Casida 1958b). Therefore, the hydrolysis in mammals at the alkyl-phosphate bond appears to occur with excessive dosages that cannot be detoxified through the system hydrolyzing the aryl-phosphate bond.

Alkyl-phosphate hydrolysis, while possibly contributing to the differences in toxicity of these compounds to insects and mammals, does not seem to be a factor in the wide range of selectivity of the different dialkyl aryl phosphorothioates toward mammals. Other factors such as hydrolytic stability, partitioning properties in respect to fat accumulation, and selectivity in rate and type of enzyme inhibition are probably more important in this respect.

A more complex hydrolytic metabolism of phosphates in mammals than in insects has been demonstrated previously with the selective phosphorodithioate insecticide malathion, *O,O*-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate (March *et al.* 1956). A larger number of hydrolytic metabolites were obtained from mice and chickens than from cockroaches and it was postulated that they were formed by progressive hydrolysis of the ethyl ester moieties. It is possible that alkyl-phosphate hydrolysis is important here also as the occurrence of this phenomenon has been demonstrated with a similar insecticide, Am. Cyanamid 12880 *O,O*-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate (Dauterman *et al.* 1957). Alkyl-phosphate hydrolysis has also been postulated in the *in vivo* metabolism of Dipterex, *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate (Robbins *et al.* 1956, Arthur & Casida 1958), and has been demonstrated with Bayer 21/199, *O,O*-diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate (Krueger *et al.* 1958).

Differences in metabolism of organophosphates between cockroaches and rats are also shown by the proportion of oxidized to non-oxidized metabolites recovered in the hydrolysis products. The dimethyl phosphorothioates were oxidized at a more rapid rate in rats than the diethyl derivatives. In cockroaches the reverse was generally true. It was not ascertained if the oxidation of the phosphorothioate grouping to the phosphate occurred before or after hydrolysis.

REFERENCES CITED

Aldridge, W. N. 1954. Inhibition of cholinesterase by organophosphorus compounds and reversal of this reaction: mechanism involved. Chem. and Indust. **1954**: 473–6.

Arthur, B. W., and J. E. Casida. 1958. Biological activity of several *O,O*-dialkyl alpha-acyl-oxyethyl phosphonates. Jour. Agric. Food Chem. **6**: 360–5.

Bowman, J. S., and J. E. Casida. 1957. Metabolism of the systemic insecticide *O,O*-diethyl S-ethylthiomethyl phosphorodithioate (Thimet) in plants. Jour. Agric. Food Chem. **5**: 192–7.

Casida, J. E. 1958. Isotope exchange for synthesis of phosphorothioate insecticides. Jour. Amer. Chem. Soc. (Manuscript in preparation)

Comar, C. L. 1955. *Radioisotopes in Biology and Agriculture.* McGraw-Hill, New York.

Dauterman, W. C., J. B. Knaak, T. Kowalczyk, and J. E. Casida. 1957. Metabolism and residues in cattle associated with the antimyiatic agent CL 12880 (*O,O*-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate). 5th Ann. Meeting, Ent. Soc. America, Memphis, December, 1957.

Fletcher, J. H., J. C. Hamilton, I. Hechenbleikner, E. I. Hoegberg, B. J. Sertl, and J. T. Cassaday. 1950. The synthesis of parathion and some closely related compounds. Jour. Amer. Chem. Soc. **72**: 2461–4.

Frawley, J. P., E. C. Hagan, and O. G. Fitzhugh. 1952. A comparative pharmacological and toxicological study of organic phosphate anticholinesterase compounds. Jour. Pharmacol. and Exptl. Therap. **105**: 156–65.

Fukuto, T. R., and R. L. Metcalf. 1956. Structure and insecticidal activity of some diethyl substituted phenyl phosphates. Jour. Agric. Food Chem. **4**: 930–5.

Kosolapoff, G. M. 1950. *Organophosphorus Compounds.* Wiley, New York.

Krueger, H. R., and J. E. Casida. 1957. Toxicity of fifteen organophosphorus insecticides to several insect species and to rats. Jour. Econ. Ent. **50**: 356–8.

Krueger, H. R., J. E. Casida, and R. P. Niedermeier. 1958. Metabolism and residues associated with dermal application of Co-Ral (*O,O*-diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate) to rats, a goat and a cow. Jour. Agric. Food Chem. (Manuscript submitted)

Louloudes, S. J., J. N. Kaplanis, and C. C. Roan. 1956. The synthesis of radioactive Diazinon using P^{32}. Jour. Organ. Chem. **21**: 685–6.

March, R. B., T. R. Fukuto, R. L. Metcalf, and M. G. Maxon. 1956. Fate of P^{32}-labeled malathion in the laying hen, white mouse, and American cockroach. Jour. Econ. Ent. **49**: 185–95.

Plapp, F. W., and J. E. Casida. 1958a. Ion exchange chromatography for hydrolysis products of organophosphate insecticides. Analyt. Chem. **30**: 1622–4.

Plapp, F. W., and J. E. Casida. 1958b. Bovine metabolism of organophosphate insecticides. II. Metabolic fate of *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate in rats and a cow. Jour. Agric. Food Chem. **6**: 662–70.

Robbins, W. E., T. L. Hopkins, and G. W. Eddy. 1956. The metabolism of P^{32}-labeled Bayer L 13/59 in the bovine body. Jour. Econ. Ent. **49**: 801–6.

Robbins, W. E. 1957. Personal communication.

Influence of Seeding Density and Row Spacings on the Resistance of Spring Wheats to the Wheat Stem Sawfly[1]

P. Luginbill, Jr., *Entomology Research Division*, and F. H. McNeal, *Crops Research Division*, *Agricultural Research Service, U.S.D.A.*[2]

Abstract

In the spring-wheat varieties solid-stemmed Rescue and hollow-stemmed Thatcher infestation and cutting by the wheat stem sawfly (*Cephus cinctus* Nort.) decreased as the seeding density increased and row spacing decreased. The decrease in cutting was due in part to a lower infestation, which was in turn associated with a decrease in moisture in stems, diameter of stems, and plant height at peak of oviposition. Apparently sawflies selected the larger, more succulent stems in the lighter seedings for oviposition.

Environmental factors are known to influence the resistance of wheat to the wheat stem sawfly (*Cephus cinctus* Nort.). Roberts (1954) found that soil conditions influenced larval survival within the host. Luginbill & McNeal (1954) reported that the use of certain fertilizers increased the amount of sawfly cutting on both spring and winter wheat varieties. Kemp (1934) showed that stem solidness is associated with sawfly resistance in Rescue. Platt (1941) showed that solidness of stem is affected by variations in light, temperature, and moisture.

In 1954 and 1955 experiments were conducted in infested fields near Choteau, Mont., to determine the effect of density of seeding and row spacings on activities of the wheat stem sawfly and also on resistance of spring wheat to sawfly infestation and cutting.

Materials and Methods.—Thatcher and Rescue were the varieties grown in 1954 and Thatcher was also grown in 1955. Thatcher is a hollow-stemmed spring wheat and is very susceptible to sawfly damage. Rescue has a solid stem and is grown in Montana and North Dakota partly because of its resistance to this sawfly.

Four seeding densities were made. The basic density was 10.5 grams per 15-foot row, the amount of seed planted when seeding at the rate of 1 bushel per acre in 12-inch spacings. The other densities were 5.25, 21, and 42 grams per 15-foot row. Row spacings of 3 and 12 inches in 1954 and 6 and 12 inches in 1955 were used at each seeding density.

A randomized-block design, with four replications, was used in all tests. Each plot was 6 feet wide and 15 feet long, and was at right angles to an adjacent sawfly-infested field of stubble.

Sawfly-cutting data from each plot were based on the average number of stems cut in six 2-foot samples taken at random. The percentage infestation for each plot was obtained by examining 25 stems taken at random from the same samples. Moisture percentages 1 week before harvest and at the peak of oviposition, which was July 15 in 1954 and 1955, were determined by weighing 1 pound of green stems from each plot before and after drying.

Plant height was the average of measurements taken from the middle and both ends of each plot. Stem diameter was determined at the time of oviposition by measuring the middle of the third internode from the top of five stems at random in each plot.

Stem-solidness ratings were based on readings from 10 stems taken at random from each plot. Each internode was cut in cross-section and rated on a scale from 1 for complete hollowness to 5 for complete solidness. Ratings of internodes were averaged and then added to obtain a solidness reading for the stem.

The data were evaluated statistically at the Montana State College Statistical Laboratory.

Results.—Sawfly and agronomic data are presented in table 1, and correlation coefficients comparing these data in table 2. The amount of sawfly cutting is shown in figure 1.

Analyses of per cent cutting confirm that Rescue is more resistant than Thatcher. The most cutting occurred in plots having wide spacings between rows. *B* and *C* in figure 1 show sawfly cutting in Thatcher at 12- and 6-inch spacings, respectively.

Seeding density was also significant, the more densely seeded rows having the least cutting. *A*, *B*, and *D* (figure 1) show sawfly cutting in Thatcher at basic and two and four times basic density, respectively, with 12-inch spacing. However, the effect of density of seeding was more noticeable with 3-inch spacing.

Spacing and density of seeding affected sawfly cutting in both varieties in a similar manner.

Seeding density had a more pronounced effect on cutting in heavily seeded plots in 1955 than in 1954 on Thatcher wheat, but spacing between rows had the same effect in both years.

Plots having wide spacing between rows had the most stems cut and the more densely seeded rows the fewest.

Spacing between rows and the seeding density influenced sawfly infestation in much the same manner, most plots with the wide spacings having the highest and those with the more densely seeded rows the lowest percentage infestation. However, seeding density had practically no effect on infestation in 1954 at 12-inch spacing between rows.

The amount of stem solidness was greatest in plots with the widest spacings. The more densely seeded rows also had the least stem solidness.

Plots with the lighest seeding density and widest spacings between rows had the highest stem moisture both at the peak of oviposition and 1 week before harvest. Greater plant height and stem diameters at peak oviposition were also obtained from most of these plots.

The positive correlation coefficients obtained when cutting percentages were compared with infestation percentages indicate that sawfly cutting is closely associated with infestation. Low infestation percentages cannot account for all the reduction in cutting that accompanied the different seeding densities and spacings. For example, in 1954 Thatcher planted at four times the basic density

[1] Accepted for publication June 2, 1958. Paper No. 406 Journal Series of Montana Agricultural Experiment Station, published with approval of the Director.

[2] The authors acknowledge the valuable assistance of B. Ostle of the Montana Agricultural Experiment Station and M. A. Berg of the Crops Research Division.

Table 1.—Sawfly and agronomic data on Thatcher and Rescue wheat grown at different row spacings and seeding densities at Choteau, Montana, in 1954 and 1955.

Spacing (Inches Between Rows)	Grams of Seed per Row	Per Cent of Stems Cut	Number of Stems Cut	Per Cent of Stems Infested	Stem—Solidness Rating	Plant Height at Oviposition Peak (In.)	Stem Diameter at Peak Oviposition (mm.)	Per Cent of Stem Moisture	
								At Peak Oviposition	1 Week Before Harvest
				1954					
Thatcher									
3	42	0.3	0.4	6.7	—	14.3	1.17	57.2	17.3
	21	8.5	4.1	50.0	—	16.0	1.50	58.0	14.2
	10.5	60.9	19.1	88.3	—	18.9	1.60	60.2	14.9
	5.25	83.5	27.4	99.2	—	18.5	2.26	63.7	30.7
12	42	42.0	30.4	93.3	—	18.7	1.95	65.8	20.0
	21	86.1	47.7	99.2	—	18.2	2.20	66.8	35.7
	10.5	93.5	59.4	100	—	18.1	2.49	71.3	35.7
	5.25	93.8	55.4	100	—	15.1	2.74	70.8	38.9
Rescue									
3	42	0.8	0.9	18.3	2.3	15.2	1.32	59.0	24.4
	21	4.5	2.7	53.3	3.5	17.7	1.95	60.0	22.9
	10.5	12.0	4.2	81.7	3.9	18.0	1.91	62.0	25.0
	5.25	33.9	9.5	93.4	4.4	18.2	2.34	66.0	35.0
12	42	25.3	17.9	91.7	3.8	17.9	1.98	68.3	30.8
	21	36.6	19.1	95.0	4.0	17.3	2.38	68.8	34.3
	10.5	53.2	31.4	91.7	4.4	17.1	2.66	74.0	42.3
	5.25	57.2	32.7	96.7	4.5	17.4	2.70	72.8	41.9
				1955					
Thatcher									
6	42	1.6	2.8	3.5	—	12.4	1.54	62.6	30.3
	21	17.2	14.6	33.8	—	13.6	1.84	61.7	29.2
	10.5	76.2	32.2	81.9	—	16.1	2.44	63.3	35.6
	5.25	77.0	27.6	85.0	—	14.8	2.62	61.1	37.8
12	42	6.2	12.7	12.1	—	13.7	1.76	62.8	31.9
	21	50.4	45.5	69.1	—	16.4	2.35	61.2	30.9
	10.5	81.8	46.9	88.2	—	15.6	2.65	66.8	37.3
	5.25	79.6	44.3	87.1	—	15.5	2.95	65.7	38.0

in 12-inch spacings was infested 93.3% and cut only 42.0%, whereas in 3-inch spacings plots seeded at twice the basic rate were infested 50% and cut only 8.5% (table 1). Davis (1955) reported that when the moisture content of plants is less than 30% larval survival becomes difficult. Data taken 1 week before harvest showed that moisture content of stems in these examples and several other plots seeded at the heavier rates with narrow spacings were below 30%, indicating that there also may have been some relation between stem moisture content and larval survival in these plots.

There is also a close correlation between percentage of sawfly cutting and stem moisture content about 1 week before harvest.

The positive correlations obtained between percentage sawfly cutting and stem solidness were not expected. Stem solidness is usually associated with resistance to cutting. The low cutting of Rescue in heavier seedings was probably associated with other factors in view of the decrease in solidness with heavier rates (table 1).

Positive correlation coefficients obtained in comparisons of plant height, stem diameter, and stem moisture content at oviposition, with percentage infestation indicate that adult sawflies selected the larger, more succulent stems for oviposition.

Seeding densities that gave satisfactory sawfly control in these investigations would not be practical for use in wheat fields. However, the fundamental knowledge gained from this study may be of value in development of sawfly control measures.

Table 2.—Correlation coefficients comparing percentage of sawfly infestation and cutting with various agronomic factors.

Comparison	Year		Variety		Spacing			Seeding Density[a]			
	1954 (N-64)	1955 (N-32)	Thatcher (N-64)	Rescue (N-32)	3 In. (N-32)	6 In. (N-16)	12 In. (N-48)	42	21	10.5	5.25
								(N-24)			
Infestation versus:											
Cutting	0.706**	0.977**	0.899**	0.666**	0.697**	0.975**	0.635**	0.856**	0.737**	0.437**	0.091
Height	.645**	.805**	.740**	.606**	.841**	.859**	.676**	.928	.670**	.341	.433
Stem diameter	.766**	.917**	.218	.796**	.792**	.938**	.483**	.735**	.646**	.277	.176
Per cent moisture at peak oviposition	.691**	.596**	.492**	.736**	.654**	−.151	.548**	.645**	.608**	.506*	.335
Cutting versus:											
Per cent moisture 1 week before harvest	.569**	.601**	.561**	.907**	.295	.628**	.433**	.123	.636**	.353	.062
Stem solidness (Rescue)	.593**	—	—	.593**	.545**	—	.291	.586	.433	.827*	−.513

[a] Grams per 15-foot row.
* Significant at P = 0.05.
** Significant at P = .01.

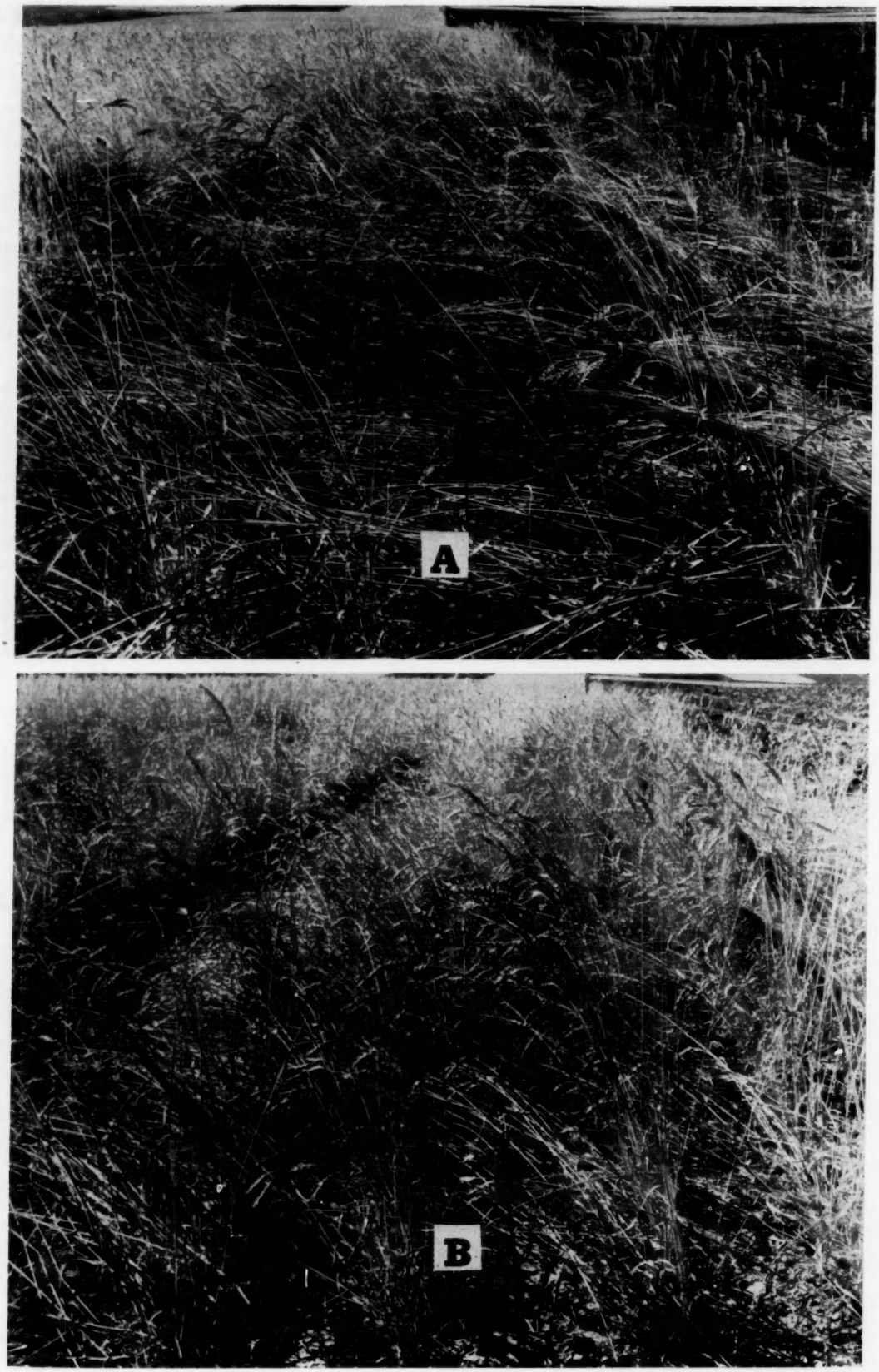
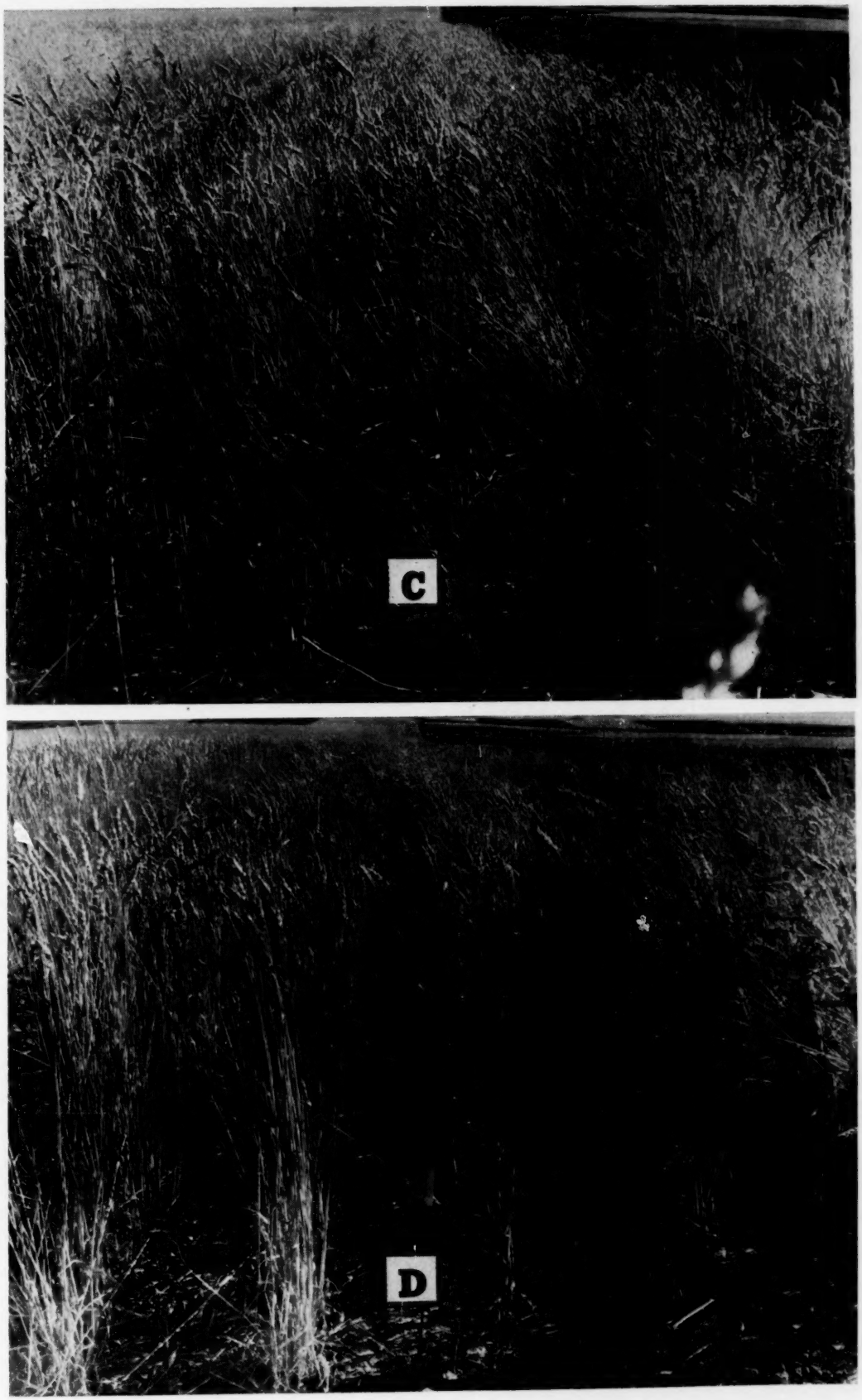


FIG. 1.—Sawfly cutting in Thatcher wheat in relation to seeding density and spacing between rows: *A*, at basic density (10.5 grams per 15-foot row) and 12-inch spacing; *B*, at twice basic density and 12-inch spacing; *C*, at twice basic density and 6-inch spacing; and *D*, at four times basic density and 12-inch spacing.


C
D

REFERENCES CITED

Davis, E. G. 1955. Status of the wheat stem sawfly in the United States in 1954. North Dakota Agric. Expt. Sta. Bimo. Bull. 17(5): 171–5.

Kemp, H. J. 1934. Studies on solid stem wheat varieties in relation to wheat stem sawfly control. Sci. Agric. 15: 30–38.

Luginbill, P., Jr., and F. H. McNeal. 1954. Effect of fertilizers on the resistance of certain winter and spring wheat varieties to the wheat stem sawfly. Agron. Jour. 46: 570–3.

Platt, A. W. 1941. The influence of some environmental factors on the expression of the solid stem character in certain wheat varieties. Sci. Agric. 22: 139–51.

Roberts, D. W. A. 1954. Sawfly resistance in wheat. I. Types of resistance. Canadian Jour. Agric. Sci. 34: 582–97.

Treatment Time with Dow ET-57 for Cattle Grub Control[1]

L. L. WADE[2] and R. W. COLBY[3]

ABSTRACT

Grub control in cattle given Dow ET-57 (Trolene[4]) and Dow ET-57 with purified phenothiazine during different months after heel fly activity ranged from 80 to 100%. There were no appreciable differences in the control obtained with dosages of 75 and 100 mg./kg. of Dow ET-57 or with 75 mg./kg. Dow ET-57 with 100 mg./kg. purified phenothiazine. Also, there were no apparent differences between the grub control obtained from treating cattle as early as May and those treated at anytime during the subsequent months prior to appearance of grubs on the backs.

Two species of cattle grubs, *Hypoderma lineatum* (De-Vill.) and *Hypoderma bovis* (L.) are reported to be the major insect pests of cattle in the United States. The losses caused by cattle grubs are estimated at more than 100 million dollars annually (Smith 1948). A greater portion of these losses is due to hide damage and excessive carcass trim that is required when cattle are slaughtered during the season that grubs are encysted in the backs of the animals. There was a loss of at least 14 million pounds of meat trimmed out of grubby cattle carcasses in the United States in 1948.

An additional undetermined amount of loss is caused by the larvae migrating within the body of the animal. This migration covers a period of 6 to 8 months prior to the encystment of grubs in the backs of the cattle. During this prolonged period the substances excreted by the larvae, the mechanical damage, and the irritation of body tissues may cause a greater degree of unthriftiness in the animal than is generally recognized.

Bishopp & Laake (1929) showed that rotenone applied under pressure as a spray would kill cattle grub larvae in the backs of cattle. This was the control measure followed for many years, although this treatment did not prevent the damaging effects by the larvae to the carcass and hide.

Recent work by various research stations has shown that some insecticides when administered internally to cattle prevented the development of cattle grub larvae. McGregor *et al.* (1954) showed that certain phosphorous compounds given to cattle infested with grubs killed the larvae encysted in the backs of the cattle but not the migrating larvae. McGregor *et al.* (1955) administered several chlorinated hydrocarbon insecticides orally to cattle. These compounds also killed the encysted larvae but not the migrating stages. Following these studies, McGregor & Bushland (1957) and Eddy & Roth (1957) working at Kerrville, Texas and Corvallis, Oregon, United States Department of Agriculture Animal Parasite Laboratories, respectively, simultaneously administered Dow ET-57 orally at a dose of 100 mg./kg. of body weight to cattle infested with larvae of *H. lineatum* and *H. bovis*. These cattle were carefully observed during the following grub season and not a single grub appeared in the backs of the treated cattle. Further work by entomologists of the U.S. Department of Agriculture and The Dow Chemical Company showed that a single oral dose of Dow ET-57, when administered prior to the time that cattle grubs encysted in the backs of the cattle, destroyed the migrating stages of the larvae. Since it was established that a single oral dose of Dow ET-57 was effective against cattle grubs, further work was deemed necessary to ascertain the best time to administer this compound for the most effective results.

The following tests were designed to determine the optimum time for treatment and the dosage needed in order to prevent grubs from encysting in the backs of cattle.

METHODS AND MATERIALS.—The tests were conducted on two herds of cattle owned by the Texas State Department of Corrections, Clemens Unit, Brazoria, Texas. These two herds, totaling 240 head, consisted of Brahman and Brahman-Hereford crosses and ranged in age from yearling heifers to 7-year-old cows. Prior to treatment the cattle were weighed and neck-chained for future identification.

The cattle were divided into 23 groups for treatment. Each month for 7 months beginning in May, three untreated groups were given oral doses as follows: (1) 75 mg./kg. Dow ET-57, (2) 100 mg./kg. Dow ET-57, and (3) 75 mg./kg. Dow ET-57 plus 100 mg./kg. of purified phenothiazine. The Dow ET-57 was formulated as a 25% wettable powder and diluted with water in order that 1 ounce of drench would treat 100 pounds of body weight at the dosage required. Each cow was weighed prior to treatment and the dosages calculated on a mg./kg. basis. The first treatment was given May 3, which was approximately 1 month after the last grubs were seen in the backs of cattle in this area. The last treatments were given in November.

[1] Accepted for publication June 2, 1958.

[2] Senior Entomologist.

[3] Director of Laboratory, Agricultural Research Department, Texas Division. The Dow Chemical Company, Lake Jackson, Texas.

[4] Registered trademark of The Dow Chemical Company for *O,O*-dimethyl O(2,4,5-trichlorophenyl) phosphorothioate.

Table 1.—Grubs in cattle given oral drenches of Dow ET-57 at monthly intervals from May to November, 1956.

Date Treated	Dosage of Dow ET-57: 100 Mg./Kg. No. Head Examined	100 Mg./Kg. Grubs per Head	75 Mg./Kg. No. Head Examined	75 Mg./Kg. Grubs per Head	75 Mg./Kg. plus 100 Mg./Kg. phenothiazine No. Head Examined	75 Mg./Kg. plus 100 Mg./Kg. phenothiazine Grubs per Head	Untreated No. Head Examined	Untreated Grubs per Head
				Herd No. 1				
May 3	8	0.9	10	1.4	8	1.1		
June 8	9	1.4	6	0.3	9	1.9	13	9.0
July 3	6	0.2	7	0.1	6	0.0		
				Herd No. 2				
Aug. 3	7	9.4	6	3.3	7	3.6		
Sept. 4	9	1.8	8	1.6	7	3.0	8	17.4
Oct. 3	10	1.3	9	1.2	9	1.1		
Nov. 5	10	1.7	10	0.7	—	—		

The grub counts were made on December 31, 1956, and February 15, 1957.

RESULTS.—The average number of grubs per animal in the backs of the cattle was greatly reduced in all treatments applied from May to November, with the exception of the 100 mg./kg. treatment in August (table 1). All groups treated with Dow ET-57 at 100 mg./kg. showed 82.4% fewer grubs than the untreated animals. The reduction of grubs in the groups treated with 75 mg./kg. was 90.1%, while the groups treated with the Dow ET-57-phenothiazine combination showed a reduction of 85.4%.

In the treated groups, the number of cattle infested with grubs ranged from 0 to 50% while the control group had 86% of the cattle infested with grubs.

From these data it appears that effective grub control may be obtained by treatment anytime after the grub has entered the body of the animal. Treatments administered early in the season were equally as effective as late season treatments. Those animals treated with 100 mg./kg. of Dow ET-57 in May had an average count of 0.88 grubs per animal as compared with an average of 9.0 grubs per head in the untreated animals (90.2% reduction). The same dosage in November had an average of 1.7 grubs per head as compared with 17.38 grubs in the controls (91.2% reduction). Using a dose of 75 mg./kg. in May and June resulted in reductions of 84.5% and 96.4%, respectively, as compared with a 96.0% reduction in the November treatment (table 2). With the exception of the August treatment with 100 mg./kg., which gave only 45% reduction, there was no appreciable difference in the three treatments. This indicates that a dose somewhat less than the recommended dose of 100 mg./kg. will give a high degree of control. The addition of phenothiazine to Dow ET-57 did not increase its efficacy.

Table 2.—Reduction of grubs in cattle given oral drenches of Dow ET-57 at monthly intervals from May to November, 1956.

Date Treated	Dosage of Dow ET-57: 100 Mg./Kg.	75 Mg./Kg.	75 Mg./Kg. +100 Mg./Kg. Phenothiazine
May 3	90.2	84.5	87.6
June 8	84.0	96.4	79.0
July 3	98.1	98.5	100.0
Aug. 3	45.8	80.8	79.5
Sept. 4	89.8	90.7	82.8
Oct. 3	92.5	93.0	93.6
Nov. 5	91.2	96.0	—

The per cent reduction of grubs in the treated groups, as shown in table 2, indicates that treatments may be applied at any time during the season after all heel fly activity has ceased and before the grubs begin to encyst in the backs of the cattle.

REFERENCES CITED

Bishopp, F. C., E. W. Laake, H. M. Brundrett, and R. W. Wells. 1927. The cattle grubs or ox warbles, their biologies and suggestions for control. U. S. Dept. Agric. Bull. **1369.**

McGregor, W. S., R. D. Radeleff, and R. C. Bushland. 1954. Some phosphorus compounds as systemic insecticides against cattle grubs. Jour. Econ. Ent. **47**(3): 465.

McGregor, W. S., and R. C. Bushland. 1957. Tests with DOW-ET-57 against two species of cattle grubs. Jour. Econ. Ent. **50**(3): 246.

McGregor, W. S., R. D. Radeleff, H. V. Claborn, and R. C. Bushland. 1955. Dieldrin, aldrin, and lindane as systemic insecticides against livestock pests. Agric. Chemicals **10**(1): 34–6, 123.

Roth, A. R., and G. W. Eddy. 1957. Tests with DOW-ET-57 against cattle grubs in Oregon. Jour. Econ. Ent. **50**(3): 244.

Smith, H. R. 1948. Cattle grub eradication. Losses in marketing stock. Nat'l. Livestock Loss Prevention Board Report, **1948:** 28–38.

The Toxicity of Certain Alkoxybenzyl Esters of Chrysanthemumic Acid in Comparison with Allethrin and Pyrethrins as House Fly Sprays[1]

W. A. GERSDORFF and P. G. PIQUETT, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

ABSTRACT

When tested as space sprays on the Campbell turntable against house flies (*Musca domestica* L.), the *m*-isopropoxybenzyl, *m*-allyloxybenzyl, *m*-ethoxybenzyl, *o*-allyloxybenzyl, and *m*-propoxybenzyl esters of *dl-cis-trans*-chrysanthemumic acids were, respectively, about 22, 14, 9, 6 and 3% as toxic as allethrin, and about 68, 43, 29, 16, and 10% as toxic as pyrethrins at the 50% mortality level. Above this level the ratios were higher. The *o*-ethoxybenzyl ester was but slightly toxic and the *p*-allyloxybenzyl, *p*-propoxybenzyl, and *p*-ethoxybenzyl esters were practically nontoxic at 30 mg. per milliliter. The knockdown value of the esters was much inferior to that of allethrin or pyrethrins.

Screening tests against the house fly, *Musca domestica* L., with a number of substituted benzyl esters of *dl-cis-trans*-chrysanthemumic acid,[2] were reported by Piquett & Gersdorff (1958). Some of the most promising compounds were selected for more precise estimation of relative toxicity. Other compounds, though of low toxicity, were also selected because of their chemical relationship. This paper reports the results obtained with alkoxybenzyl esters.

MATERIALS AND METHODS.—The samples of the esters were of high purity.

Allethrin and pyrethrins were used as standards of comparison. The sample of allethrin was of 93.4% purity as determined by the hydrogenolysis method. The sample of pyrethrins was the unrefined extractive in which 54.2% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method.

After preliminary tests, sprays of the more toxic esters were prepared in refined kerosene (Deobase) at concentrations selected to cause a range of mortalities from high to low. Sprays of the esters of low toxicity were prepared in the same solvent at a single, relatively high concentration.

Knockdown and mortality of house flies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain were determined in replicated tests by the Campbell turntable method. The tests were made in three series, each on a different group of six populations of flies. All sprays in a series were tested simultaneously and but once on each population in the group. Approximately 113 flies, averaging 2 to 3 days in age, were used in each test in series 1, 106 of the same age in series 2, and 109 averaging $1\frac{1}{2}$ to $2\frac{1}{2}$ days in series 3.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis essentially as described by Finney (1952).

KNOCKDOWN AND MORTALITY RESULTS.—The knockdown and mortality data are summarized in table 1. All the toxic esters possessed knockdown value, those sprays which caused very high mortality causing practically complete knockdown.

EVALUATION OF RELATIVE TOXICITY.—Provisional individual regression lines were fitted graphically to the mortality data in series 1 and 2 and for the standards in series 3. It appeared that the data for the new synthetic compounds in series 1, more closely related structurally,

Table 1.—Knockdown and mortality of house flies caused by kerosene sprays containing certain synthetic esters of chrysanthemumic acid.

Material	Concentration (Mg./Dl.)	Per Cent: Knockdown in 25 Minutes	Per Cent: Mortality in 1 Day
Series 1. Six replicates			
Chrysanthemumic acid, *m*-allyloxybenzyl ester	2000	100	95.4
	1333	99.9	88.6
	888.9	99.7	76.4
	592.6	98.0	58.9
	395.1	92.1	29.6
	263.4	46.7	10.9
m-ethoxybenzyl ester	2000	100	85.4
	1333	99.5	74.9
	888.9	99.4	56.3
	592.6	95.8	35.8
	395.1	84.6	13.8
	263.4	52.2	4.2
m-isopropoxybenzyl ester	888.9	99.6	92.9
	592.6	98.7	81.7
	395.1	77.4	58.7
	263.4	74.9	31.9
	175.6	28.8	10.3
m-propoxybenzyl ester	3000	76.1	67.3
	2000	27.8	38.6
	1333	23.6	16.4
	888.9	30.1	7.4
	592.6	19.2	2.3
Allethrin	214.1	100	92.2
	142.8	100	78.8
	95.17	100	58.6
	63.45	100	39.3
	42.30	99.9	23.4
	28.20	99.9	7.1
Pyrethrins	759.4	100	89.8
	506.3	100	79.7
	337.5	100	69.2
	225.0	100	46.4
	150.0	100	30.2
	100.0	100	13.3
Series 2. Six replicates			
Chrysanthemumic acid *o*-allyloxybenzyl ester	3000	99.6	98.0
	2000	99.3	92.1
	1333	85.4	72.2
	888.9	79.3	38.2
	592.6	56.9	7.2
	395.1	41.8	2.5

[1] Accepted for publication June 2, 1958.

[2] These esters were synthesized by W. F. Barthel, R. T. Brown, and B. H. Alexander of this Division.

Table 1.—(*Continued*)

Material	Concentration (Mg./Dl.)	Per Cent: Knockdown in 25 Minutes	Per Cent: Mortality in 1 Day
Allethrin	214.1	100	93.0
	142.8	100	84.6
	95.17	100	68.7
	63.45	100	53.4
	42.30	99.6	31.7
	28.20	99.8	12.3
Pyrethrins	759.4	100	90.5
	506.3	100	88.0
	337.5	100	77.2
	225.0	100	58.3
	150.0	100	46.7
	100.0	100	30.4
	66.67	100	19.0
Series 3. Six replicates			
Chrysanthemumic acid, *p*-allyloxybenzyl ester	3000	0.5	1.5
o-ethoxybenzyl ester	3000	68.8	9.1
p-ethoxybenzyl ester	3000	3.1	0.5
p-propoxybenzyl ester	3000	2.1	1.2
Allethrin	214.1	100	81.9
	142.8	100	64.0
	95.17	100	39.9
	63.45	100	19.7
	42.30	99.9	5.1
	28.20	98.9	2.5
Pyrethrins	759.4	100	81.4
	506.3	100	70.0
	337.5	100	50.8
	225.0	100	28.2
	150.0	100	20.4
	100.0	100	6.9
	66.67	100	1.4

could be adequately represented by parallel lines steeper than that for allethrin, whereas the rest of the data required individually fitted lines. The statistical procedures based on this hypothesis were followed to obtain the weighted regression equations, and subsequent analysis of chi-squares gave no evidence that the data could not be so represented. However, heterogeneity factors were required in the calculation of variances in some of the analyses. These factors were 3.496 for the generalized group in series 1, 3.967 for the benzyl ester and 2.318 for pyrethrins in series 2, and 3.514 for pyrethrins in series 3. Factors were not required in the other four analyses.

The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. The standard errors (s_b) of the regression coefficients are also given.

		s_b
Series 1:		
Chrysanthemumic acid, *m*-allyloxybenzyl ester	$Y=3.4315X-4.4375$	0.095
m-ethoxybenzyl ester	$Y=3.4315X-5.0177$	.095
m-isopropoxybenzyl ester	$Y=3.4315X-3.7374$	.095
m-propoxybenzyl ester	$Y=3.4315X-6.5805$	.095
Allethrin	$Y=3.1071X-0.8774$	.086
Pyrethrins	$Y=2.6937X-1.4157$	.081
Series 2:		
Chrysanthemumic acid, *o*-allyloxybenzyl ester	$Y=5.0077X-10.1245$	0.262
Allethrin	$Y=2.9277X-0.2828$	.087
Pyrethrins	$Y=2.2003X+0.1053$	.100
Series 3:		
Allethrin	$Y=3.4833X-2.1646$	0.099
Pyrethrins	$Y=2.7436X-1.9420$	.144

From the equations in series 1 and 2, LC-50's were determined, and evaluations of relative toxicity were then obtained as the inverse ratio of the relevant pair of LC-50's. The estimations together with their standard errors are given in table 2.

In series 3, because of the low mortalities caused by the

Table 2.—Toxicity relative to allethrin and pyrethrins of some synthetic esters of chrysanthemumic acid.

Material	Series	LC-50[a] (Mg./Dl.)	Per Cent Toxicity Relative to—[b]: Allethrin	Per Cent Toxicity Relative to—[b]: Pyrethrins	Per Cent Range in Ratio to—[c]: Allethrin	Per Cent Range in Ratio to—[c]: Pyrethrins
Chrysanthemumic acid, *o*-allyloxybenzyl ester	2	1048 ±27	6.08±0.19	16.00± 0.69	3.6–10.4	6.1– 42.0
m-	1	563 ±17	13.84±0.47	42.79± 1.51	12.3–15.5	31.6– 57.9
p-	3	—	0.9	1.8	—	—
o-ethyoxybenzyl ester	3	—	1.6	3.7	—	—
m-	1	831 ±24	9.38±0.32	28.99± 1.02	8.4–10.5	21.4– 39.2
p-	3	—	0.7	1.3	—	—
m-isopropoxybenzyl ester	1	352 ±11	22.15±0.77	68.47± 2.44	19.7–24.8	50.6– 92.6
m-propoxybenzyl ester	1	2370 ±80	3.29±0.12	10.16± 0.40	2.9– 3.7	7.5– 13.7
p-	3	—	0.9	1.7	—	—
Allethrin	1	77.91± 1.30	100	309.1 ± 7.8	100	256 –373
	2	63.74± 1.18	100	263.1 ±10.3	100	172 –404
Pyrethrins	1	240.8 ± 4.6	32.35±0.82	100	39.0–26.8	100
	2	167.7 ± 5.8	38.01±1.49	100	58.3–24.8	100

[a] For the compounds for which no LC-50's are given, the equieffective concentrations of the standards are, in the order in which the compounds are named: Of allethrin, 27.2, 47.2, 20.4, and 25.6, and of pyrethrins, 54.9, 110.6, 38.3, and 51.0 mg. per deciliter.
[b] At the 50% mortality level.
[c] Between the 5 and 95% mortality levels.

benzyl esters at the single concentration, no equations were determined for these compounds. The regression equations were determined for allethrin and pyrethrins, and the relative toxicities of the benzyl esters were estimated simply by comparison of the actual concentration of their sprays with the equally effective concentrations of each standard as determined from their equations.

The regression lines determined for the benzyl esters appear regularly to be steeper than those for allethrin and therefore those for pyrethrins. Ratios of comparison with those standards will consequently increase between low and high mortality levels. Such ratios were estimated from LC-5's and LC-95's determined directly from the equations and are also given in table 2 to indicate the approximate range possible.

DISCUSSION.—The most toxic of the new compounds were the *m*-isopropoxybenzyl, the *m*-allyloxybenzyl, and the *m*-ethoxybenzyl esters; they were, respectively, about 22, 14, and 9% as toxic as allethrin, and about 68, 43, and 29% as toxic as pyrethrins. The *o* and *p* isomers of the allyloxybenzyl and ethoxybenzyl esters, the only complete groups of isomers tested, were much less toxic than the *m* derivatives, the *o* derivative being more toxic than the *p*. For this last conclusion regarding the ethoxybenzyl derivatives it is necessary to go to table 1. The three *p* derivatives were practically nontoxic at the concentration tested. With the two propoxybenzyl esters the *m* position was also of decided advantage over the *p* position. A similar conclusion may be made regarding the same positions with the two methoxybenzyl esters and with the two *sec*-butoxybenzyl esters from the results in screening tests reported by Piquett & Gersdorff (1958). When the 3-carbon side chain in the *m*-propoxybenzyl ester was altered to the iso or to the allyl arrangement, the toxicity of the compound was greater.

The knockdown value of these compounds is much inferior to that of allethrin or pyrethrins.

REFERENCES CITED

Finney, D. J. 1952. Probit analysis, 318 pp. Cambridge.

Piquett, P. G., and W. A. Gersdorff. 1958. Screening tests with certain synthetic compounds for toxicity to house flies when applied as space sprays. Jour. Econ. Ent. 51(6): 791–3.

Latent Period Phenomena in Transmission of Sugar Beet Yellow-Net Virus by Green Peach Aphids[1]

EDWARD S. SYLVESTER[2]

ABSTRACT

Using the leaf cage technique in transmission tests of sugar beet yellow-net virus by green peach aphids, *Myzus persicae* (Sulz.), the following conclusions were reached: (1) A low percentage of the insects (1.1%) acquired virus if the cage was left in contact with a virus source leaf for 1 hour. Maximum acquisition (approximately 25% of the insects proving to be infective) occurred when the cage was left in place for a 48-hour period. (2) When leaf cages, containing two aphids which had been reared on virus source plants, were placed for 1 hour on healthy test plants, approximately 4% were infected. Increasing the test feeding access time increased the probability of transmission up to a maximum of approximately 25% with periods of 24 or 48 hours in duration. (3) Analysis of the results of serial transmission experiments with single aphids allowed a variable acquisition access period and subsequent sequential test feeding access periods each approximately 24 hours in length until death of the insects, lead to the conclusion that there was a measurable latent period averaging 3.5 days in length. In this analysis, the latent period was defined as the average time (in days) in excess of the acquisition threshold period required by the aphids to begin virus transmission.

In 1949 (Sylvester) experimental results were published which led to the conclusion that there was no latent period in the transmission of sugar beet yellow-net virus by green peach aphids, *Myzus persicae* (Sulzer). It is the intent of this paper to re-examine and modify this conclusion.

MATERIALS AND METHODS.—*General.*—The yellow-net virus used was a derivative of the original source (Sylvester 1949). Sugar beets (*Beta vulgaris* L.) used to maintain virus and at times aphids, and to provide test plants were of the variety U.S. 15. Test plants were sown as seed in pasteurized sand or vermiculite and later transplanted into pasteurized soil in 3-inch clay pots. Seedling test plants normally were used in the cotyledon stage, but occasionally 2 to 3 leaves developed before use.

Aphids were maintained as noninfectives on both smooth leaf mustard (*Brassica juncea* Coss) and sugar beet plants. Large groups of aphids were collected by aspiration, while smaller groups and individuals were sorted with a camel's-hair brush. Acquisition as well as test access times were done using leaf cages (Sylvester 1956). Routine spraying with nicotine and soap solution helped minimize unwanted aphid infestations.

Since the leaf cage technique lessened the danger of vector escapes, many experiments were done within the greenhouse where test plants were also incubated. In some cases if high temperatures were likely to occur, test feeding access periods were done in the laboratory.

Inoculation Threshold Period.—To establish the inoculation threshold period using the leaf cage technique, the following procedure was used successfully on five different occasions. Mature apterae or late instar nymphs, collected by aspiration from colonies which had been reared

[1] Accepted for publication June 2, 1958.

[2] Associate Entomologist, Department of Entomology and Parasitology, University of California, Berkeley.

Table 1.—Length (mean ± standard deviation) of the serial test feeding used in connection with the various acquisition access periods in the study of the latent period of the yellow-net virus transmission by the green peach aphid.

Acquisition Access Period (Hours)	Test Feeding Period	
	N[a]	Length[b]
1	101	23.9±0.53
2	106	23.8±0.69
4	109	23.5±1.47[c]
8	105	23.0±2.17[c]
24	81	24.0±0.47
48	88	24.0±0.16

[a] N is the number of test feedings intervals recorded.
[b] The intended length of each test feeding was 24 hrs.
[c] The relative high variation found in this case was due to a gradual shortening the test feeding intervals in order to eventually allow all of the insects to be transferred at the same time.

on virus source plants, were sorted into small leaf cages, two per cage. After sorting, the cages were attached to a series of 140 healthy beet plants, one cage per plant. At random, at each of the following intervals, 20 cages were removed: 1, 2, 4, 6, 8, 26, and 48 hours. In addition, 20 cages, none of which contained insects, were attached to 20 beet plants. In two of the trials, the insects which were removed at the end of 1 hour, were placed on another series of test plants and allowed a 2-hr test access time. The insects which initially had 2 hours of test access time were moved to a second series of test plants for a period of 1 hour. Four such reciprocal cycles were made, so that in each of the two trials, the same insects had access to three sets of 20 healthy plants for 1 hour and three sets of 20 plants for a 2-hour test access period.

Acquisition threshold period and latent period.—In general, the following procedure was used in the latent period and acquisition threshold period tests. A sample of apterous aphids (late instar nymphs or apterae) was obtained from a noninfective source by aspiration, and individually sorted into 30 leaf cages. Twenty of the cages contained 15 to 20 individuals, and 10 of the cages contained 10 individuals. The 10 cages of 10 aphids per cage were placed on healthy test plants, one cage per plant, to serve as checks. These cages were moved daily to new beet plants until all insects died. The remaining 20 cages of aphids (15 to 20 insects per cage) were placed upon 20 yellow-net virus source plants, one cage per plant. Three cages were removed from the virus source plants at the end of each of the following intervals: 1, 2, 4, 8, and 24 hours, and the remaining cages (usually five) were removed at the end of 48 hours. The extra cages were used

Table 2.—Results[a] of trials to determine the inoculation threshold period of beet yellow-net virus when transmitted by lots of two green peach aphids using variable test access times and the leaf cage technique.

	Test Access Time in Hours							
	0	1	2	4	6	8	24	48
Per cent	0/96 0.00	10/257 3.8	18/255 7.0	9/100 9.0	15/98 15.3	19/99 19.1	25/100 25.0	25/99 25.2

[a] In ratios listed, numerator is number of plants infected; denominator, number tested. Variations in denominators (100 or 260 intended) due to plants or vector dying before termination of experiment.

in case of accidents due to breakage of the rubber band which held them in place, or excessive mortality in a particular group. The order of removal was at random. For any particular interval, the aphids from the removed cages were put into a container, and from the common pool, as rapidly as possible, a minimum of 20 aphids was picked and put into 20 cages, one aphid per cage. The leaf cages were then attached to a series of 20 test plants and subsequently moved at approximately 24-hour intervals (table 1) until death of the insects. The number of aphids tested per interval per trial varied from 20 to 45, the larger number of individuals being tested for the 24- and 48-hour acquisition access times. Several such trials were run, with some variation in technique. For example, on a few occasions, the aphids were sorted in the late afternoon prior to being placed on the virus source plants the next morning. In these cases the aphids were kept in a refrigerator during the remainder of the afternoon and night before use. On one occasion, the 4-hour acquisition access interval was not included by mistake, and in this run, the number of insects tested at the 8-, 24-, and 48-hour intervals was increased to 25, 40, and 45, respectively. However, within practical limits, the conditions of any one trial were kept as uniform as possible, with the exception of some variation in the number of individuals tested per interval, and it was attempted to distribute the other sources of variation at random among the treatments.

RESULTS.—*Inoculation threshold period.*—The results of all trials are given in table 2. Using two aphids per test plant, about 4% of the plants were infected when the vectors had 1-hour test access time. The chance of obtaining an infection gradually increased as the test access time was lengthened until 24 hours, after which interval no additional gain was noted. The difference between the results of the present work and the former (Sylvester 1949) where 15 minutes of test feeding was reported to be sufficient for inoculation, is not unreasonable if based upon the actual ability of certain insects to inoculate beet

Table 3.—Cumulative mortality table for the aphids which were used in the yellow-net latent period trials, using single insects, the leaf cage technique, and daily transfers to seedling beets.

Acquisition Access Time in Hours	Total No. of Aphids Counted	Mean Per Cent of Original Aphids Dead on Day Interval												
		0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13
1	337	5.6	36.6	59.9	80.1	87.8	91.4	94.6	97.3	98.8	100.0			
2	333	6.6	30.9	57.6	76.8	84.6	90.6	93.6	98.1	99.3	99.6	100.0		
4	315	8.6	29.8	56.2	69.8	78.1	85.1	92.0	95.8	98.1	98.7	99.0	99.6	100.0
8	326	6.4	28.2	46.6	59.2	70.2	79.1	88.0	94.1	97.2	98.4	99.6	100.0	
24	352	15.3	50.0	72.7	85.8	93.7	96.0	98.0	99.4	99.7	100.0			
48	340	19.1	48.2	71.5	85.6	93.5	96.4	97.9	98.7	99.0	99.6	100.0		

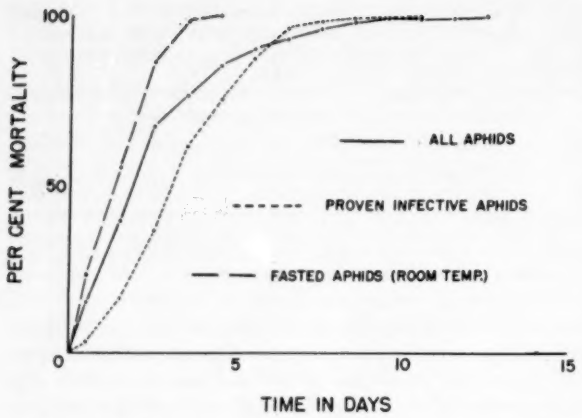


FIG. 1.—Mortality curves of various sub groups of the aphids used in trials made to determine the latent period of the yellow-net virus when transmitted by green peach aphids.

plants in relatively short test feeding times. In the former work, using 10 hours of access time on test plants, 7 of 25 insects tested (28%) were infective, whereas in the present work, using an 8-hour leaf cage test access time, an estimated 10% of the insects were infective. The general level of infectivity in the aphids in the former work was higher.

Acquisition threshold period.—During this work many of the aphids failed to survive for long series of transfers in the leaf cages, and there was variation in the death rate of the samples from trial to trial. Occasionally high temperatures occurred for brief periods in the greenhouse, and some of the later runs were made in the laboratory where the possible influence of radiant energy was considerably reduced. While the aphids lived longer under the laboratory conditions, there was no gain in the rate of transmission.

Table 4.—Results[a] of trials to determine the acquisition threshold period of the beet yellow-net virus when transmitted by single green peach aphids using the leaf cage technique and a variable acquisition.

	Acquisition Access Time in Hours						
	0	1	2	4	8	24	48
	2/960[b]	4/361	6/361	7/342	45/422	71/426	95/384
Per cent	0.2	1.1	1.7	2.0	14.0	16.7	24.7

[a] In ratios listed, numerator is number of insects infective; denominator, number tested.

[b] Check insects were tested in groups of 4, 5, or 10. One check plant was infective in each of two trials. Since lots of 5 to 10 insects were used, failure to get more than one plant infected in a series indicated that these infections were due to accidental spread in the greenhouse rather than infective source colonies of insects.

Originally it was thought that increasing the acquisition access time might increase the mortality rate in subsequent test feeding transfers. The summarized mortality data of table 3 indicates that the mortality rate in the 24- and 48-hour acquisition access periods was somewhat higher than in the other intervals. This is not so when the data are adjusted for the total time spent on beets. In general, 50% of the test insects had died by the time 72 hours of feeding on beets had been completed. The comparative mortality curves (fig. 1) for the total sample, for those aphids demonstrated to be infective, and for a sample of 98 aphids fasted under room temperature conditions, indicated that, aside from the fact that fasting increased the mortality rate, there was an apparent tendency for the infective aphids (214 cases) to die at a slower rate than the general population. This is most simply explained by the fact that since the probability of infecting a plant was less than 1, those insects which lived longest had more of a chance to demonstrate their infectivity. Presumably some infective aphids died before they could infect a plant.

From the results (table 4), in spite of a low percentage

Table 5.—Results[a] of trials to determine the amount of transmission of the yellow-net virus to sugar beets by single green peach aphids, allowed a variable acquisition access time after which they were transferred at approximately 24 hr. intervals to a series of healthy test beets until death.

Trial No.	Acquisition Access Period in Hours							
	0[b]	1	2	4	8	24	48	Total[c]
4	0/8	0/42	0/40	0/41	—	6/52	—	6/175
5	0/21	0/54	0/65	2/63	—	6/90	—	8/272
6	0/22	0/78	0/85	5/101	15/116	12/90	25/79	57/549
7	1/55	2/72	0/77	1/77	6/71	9/63	13/94	31/454
8	1/40	0/71	0/96	0/71	7/91	5/79	2/55	14/463
9	0/35	0/48	1/60	1/53	0/57	2/31	3/62	7/311
10	0/35	2/59	5/67	—	27/141	55/137	84/146	173/550
11	0/52	0/83	1/84	0/87	1/95	0/71	5/84	7/504
13	0/29	0/57	0/57	0/53	2/67	0/49	2/41	4/324
14	0/32	0/50	0/58	0/55	1/58	4/59	5/65	10/345
15	0/33	0/69	0/58	0/69	1/63	1/48	8/76	10/383
16	0/33	0/64	0/64	0/70	1/71	5/61	8/83	14/413
17	0/37	0/76	0/64	0/78	3/98	3/61	2/59	8/436
18	0/84	0/140	0/143	0/146	1/132	0/103	1/78	2/742
19	0/68	0/130	0/105	0/123	2/187	0/217	1/98	3/770
20	0/68	0/132	0/124	0/160	4/179	1/92	5/99	10/786
Total	2/652	4/1225	7/1247	9/1247	71/1426	109/1213	164/1119	364/7477

[a] In ratios listed, numerator is number of plants infected; denominator, number tested.

[b] Insects, in lots of 4, 5, or 10 insects per lot, tested on number of plants indicated by denominator, with a total of 960 insects being used.

[c] Totals in this column do not include check (0 acquisition access time).

Table 6.—Patterns[a] of yellow-net virus transmission by single green peach aphids given a variable acquisition access time, and then serially transferred at approximately 24-hour intervals to healthy beet seedlings until death.

Acquisition Access Time	Frequency
1 Hour	
− + − ×	1
− + − − − − ×	1
− − + − − ×	1
− − − − − − + ×	1
2 hours	
− − + ×	1
− − + − − ×	1
− − − + − ×	1
− − − + − + − ×	1
− − − − + − − ×	1
− − − − − + ×	1
4 hours	
+ − − − + ×	1
− + − − ×	1
− − + − − ×	1
− − + − − − − ×	1
− − − + − ×	1
− − − + − + − ×	1
− − − − + − − ×	1
8 hours	
+ ×	1
+ − − ×	2
+ − + − ×	1
+ + − − − 0 ×	1
+ + + + + + − ×	1
− + − ×	1
− + − − ×	1
− + + − − ×	1
− + − − − − ×	1
− + − − + + − − ×	1
− + + − + − + − − ×	1
− − + ×	1
− − + − − ×	1
− − + + − ×	1
− − + + + ×	1
− − + − − − − ×	1
− − + + + − ×	1
− − + + + − ×	1
− − + − − − − ×	1
− − + − − − − − ×	2
− − + − − − − − − − ×	1
− − − + ×	2
− − − + − − ×	4
− − − + + − ×	1
− − − + − + ×	1
− − − + − − + ×	2
− − − − + − ×	1
− − − − + − − ×	2
− − − − + − − − ×	1
− − − − + − + − ×	1
− − − − − + − ×	4
− − − − − + + ×	1
− − − − − − + − ×	2

Acquisition Access Time	Frequency
24 Hours	
+ ×	2
+ − ×	3
+ + ×	4
+ − − ×	4
+ − + ×	1
+ + − ×	5
+ + + ×	1
+ − + − ×	1
+ + − − ×	1
+ − + − − − ×	1
− + ×	6
− + 0 ×	1
− + − ×	4
− + + ×	1
− + − − ×	1
− + − + ×	1
− + + − ×	1
− + − + − ×	3
− + + + − ×	2
− + − − + − ×	1
− − + − ×	10
− − + + ×	3
− − + + − ×	2
− − + − − − ×	1
− − − + ×	2
− − − + − − ×	2
− − − + − + − − ×	1
− − − − + − ×	1
48 hours	
+ ×	4
+ − ×	15
+ + ×	1
+ − − ×	4
+ + − ×	2
+ + + ×	2
+ + − − ×	5
+ + + − ×	3
+ + + + ×	1
+ − − − − ×	1
+ − + − − ×	1
+ − − + − ×	2
+ − + + − ×	1
+ + + + − ×	3
+ − − − − − ×	1
− + ×	2
− + − ×	9
− + + ×	4
− + − − ×	5
− + + − ×	3
− + + + ×	2
− + − − − ×	1
− + + − − ×	1
− + + + − ×	3
− + + + + ×	1
− + − + − − ×	2
− + + + − − ×	1
− + + + 0 − ×	1
− + − − − − − ×	1
− + + + + + + ×	1
− − + ×	3
− − + − ×	4
− − + − − ×	1
− − + + − ×	2
− − − + ×	1
− − − + − − ×	1

[a] Each symbol represents a plant fed upon. Minus (−) indicates no infection, plus (+) indicates infection, (×) indicates the day the insect died, and zero (0) indicates death of the test plant before it could be read.

Table 7.—Results of analysis of data relevant to the determination of a latent period in the transmission of the sugar beet yellow-net virus by green peach aphids.[a]

Acquisition Access Period	Test Feeding Interval	Plants Infected		Plants not Infected	
		Observed	Expected[b]	Observed	Expected
48 hours	1	46	50.6	49	44.4
	2	53	48.5	37	42.5
	3	38	38.9	35	34.1
		$\chi^2=2.27$; d.f.=2; p=0.50			
24 hours	1	23	32.2	48	38.8
	2	37	31.3	32	37.7
	3	29	24.9	26	30.1
	4	17	17.7	22	21.3
		$\chi^2=7.99$; d.f.=3; p=0.05			
8 hours	1	6	11.9	39	33.1
	2	8	11.6	36	32.4
	3	15	11.6	29	32.4
	4	14	10.6	26	29.4
	5	12	9.2	23	25.9
		$\chi^2=9.48$; d.f.=4; p=0.05			

[a] Single aphids were allowed a variable acquisition access period on a virus infected beet, and then were transferred until death to a series of healthy test sugar beet seedlings at 24-hour intervals.
[b] Expected based upon number of plants infected out of number tested.

of contamination, it can be concluded that acquisition of virus increased most rapidly during the first 8 hours of acquisition access time on the virus source plant. The less rapid gain in infectivity during the 24- and 48-hour periods when compared with the 8-hour period, suggests that after the initial rapid rise there continued to be a slow increase in the number of individuals acquiring virus when access to a virus source was allowed to continue.

When these results are compared with those previously reported (Sylvester 1949) there is some difference. For example, in the former work transmission was obtained with an acquisition feeding of 5 minutes. Much of the difference was probably due to the lower level of infectivity in the present work. The proportion of plants infected using a 24-hour acquisition access and one 24-hour test access time in the present work was 5.3% (21/395), whereas in the former work, it was approximately 26% based upon 125 examples. In addition, in the previous work, a watched-timed acquisition feeding period was used for the intervals of 2, 4, 5, 10, 15, 20 and 30 minutes (weighted $\bar{x}=12.3$ min.), while in the present work all acquisition times (minimum of 1 hour) were access periods in leaf cages. In the former work, combining all the results of the mentioned intervals gave a total of eight infections with 210 insects, while in the present work, only four infective aphids out of 301 tested were obtained with the 1-hour acquisition access interval, and this was only possible under conditions of serial transfer where most of the insects had more than one opportunity to demonstrate their infectivity. Thus the relatively low level of vector infectivity combined with the effects of the leaf cage technique probably accounts for the differences between this and the former work.

Latent period.—Table 5 gives the results considered in establishing the latent period, based upon the number of plants infected in the serial transfers of individual aphids, sorted as to length of the acquisition access time and as to the individual trials. The heterogeneity due to treatment and to trial is pronounced. It is evident that a considerable number of insects tested failed to inoculate plants in the test series. This could have been due to individuals not acquiring an infective charge of virus, or death occurring before they could demonstrate their infectivity. Which of these situations prevailed, or to what proportion both were true, is not known. Some approximations could be used to estimate the proportion of each, but it is not believed that it is essential to the interpretation of these data in connection with the latent period. The remainder of the discussion is limited to those individuals which were found to be infective sometime during their test feeding sequence. The individual patterns of transmission and the frequency of their occurrence are given in table 6.

For analysis, the following assumptions were made concerning the test populations.

1. If there is no latent period, the probability of transmission will be at a maximum at the completion of the acquisition access period.

2. The length of the acquisition access period determines the level of transmission, and measurement of the latent period can only be made after completion of the acquisition period.

3. The only deviations from the expected rate of transmission relevant to the question of a latent period are those which occur in the first few days of testing.

The data were tested in the following three cases. (1) acquisition access period 48 hours, (2) 24 hours, and (3) 8 hours. The results of the analysis are given in table 7, and graphically presented in figs. 2, 3, and 4.

Use of a 48-hour acquisition access period practically eliminated the possibility of demonstrating a latent period. The results obtained with both the 24-hour and the 8-hour acquisition access periods were of more value. In these cases the chi square value was at the 5% level of

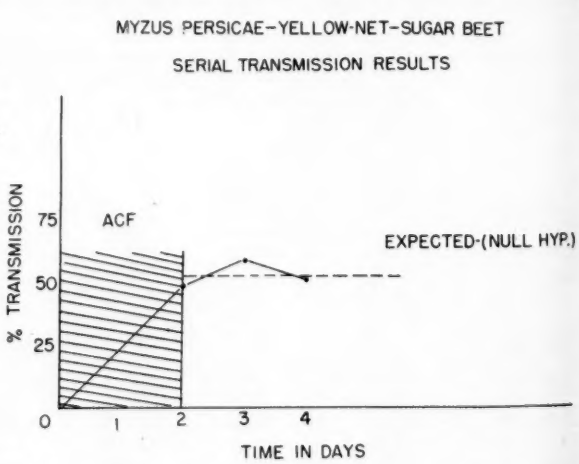


FIG. 2.—Graph of relevant results of trials made to determine the presence of a latent period in the transmission of the yellow-net virus by green peach aphids. Single aphids were allowed a 48-hour acquisition access feeding period after which they were serially transferred to test seedlings at daily intervals until death.

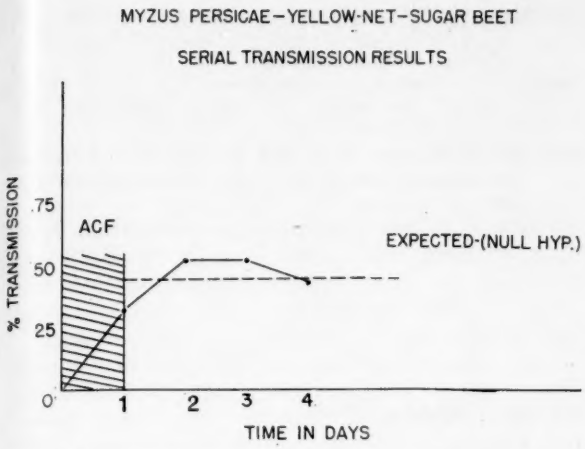


FIG. 3.—Graph of relevant results of trials made to determine the presence of a latent period in the transmission of the yellow-net virus by green peach aphids. Single aphids were allowed a 24-hour acquisition access feeding period after which they were serially transferred to test seedlings at daily intervals until death.

significance, indicating a rather strong tendency for the evidence not to support the null hypothesis, *viz.*, the variation that occurred was due to chance. Shortening the acquisition access time allowed more of the latent period to be measured.

Table 8 compares the time and frequency with which aphids given an 8-hour acquisition access period, first began virus transmission, with a distribution expected if no latent period was present. Again there is statistical support for the evidence of a latent period. Of interest is that the means of the two distributions are approximately the same. However, the observed resulted from a situation in which the probability of the event (the appearance of a new vector) was increasing (due to the latent period), while the other is the mean of a geometric series in which the probability of the event is decreasing.

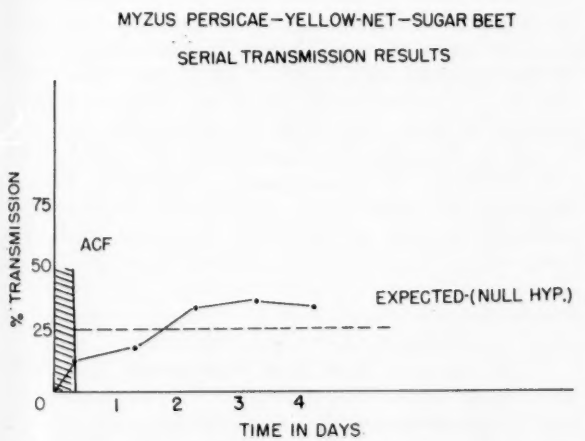


FIG. 4.—Graph of relevant results of trials made to determine the presence of a latent period in the transmission of the yellow-net virus by green peach aphids. Single aphids were allowed an 8-hour acquisition access feeding period after which they were serially transferred to test seedlings at daily intervals until death.

Table 8.—Comparison of results obtained in transmission of sugar-beet yellow-net virus by single green peach aphids with those expected under a "no latent period" hypothesis.[a]

TEST FEEDING PERIOD NUMBER	NUMBER OF APHIDS FIRST TRANSMITTING	
	Observed	Expected[b]
1	6	11.7
2	6	8.6
3	11	6.4
4–7	22	18.3
	Mean 3.6	3.9
	$\chi^2=7.07$; d.f.=2; p=<0.05	

[a] The acquisition access period was 8 hrs., and each serial test feeding access period was approximately 24 hours.
[b] Based upon 71 infections out of 274 tests.

DISCUSSION.—In contrast with leafhopper vector-virus relationships, in which latent period phenomena frequently have been well established, the question as to whether or not they exist in aphid vector-virus relationships is more pressing. With aphids there are limiting factors in many latent period investigations. Among these are (1) the relatively long period of feeding required to insure both adequate virus acquisition and adequate inoculation, (2) the relatively short life of aphids moved at frequent intervals, (3) the brevity of the latent period in many cases, and (4) the tendency for the probability of transmission to decrease as the test feeding sequence is prolonged.

Mistakingly emphasizing the importance of a minimum latent period, the author (Sylvester 1949) published the conclusion that in the transmission of sugar beet yellow-net virus by green peach aphids a definite latent period did not exist. The results indicated that there was no period of time beyond that occupied by the acquisition and inoculation threshold periods in which transmission could not be demonstrated, providing enough individuals were tested. This still may be true, but it does not mean that in properly designed serial transmission trials there will not be a delay in the development of infectivity within the test populations.

Thus the beet yellow-net virus seems to be another of the aphid-borne persistent plant viruses which possess a measurable latent period. The best known other examples of this group are potato leafroll (Smith 1931, Day 1955), pea-enation mosaic (Osborn 1935, Chaudhuri 1950, Simons 1954), filaree red-leaf (Anderson 1951), and strawberry virus 3 (Prentice & Woollcombe 1951). There are others which might be listed, but to date experimental work upon them is less detailed, or their status is somewhat uncertain, *e.g.*, carrot motley dwarf (Stubbs 1948).

REFERENCES CITED

Anderson, C. W. 1951. The insect vector relationships of the filaree red-leaf virus, with special reference to a latent-period difference between nymphs and adults in *Macrosiphum geranicola* (Lambers). Phytopath. **41**: 699–708.

Chaudhuri, R. P. 1950. Studies on two aphid-transmitted viruses of leguminous crops. Ann. App. Biol. **37**: 342–54.

Day, M. F. 1955. The mechanism of the transmission of potato leafroll virus by aphids. Australian Jour. Biol. Sci. **8**: 498–513.

Osborn, H. Y. 1935. Incubation period of pea mosaic in the aphid *Macrosiphum pisi*. Phytopath. **25**: 160–77.

Prentice, I. W., and T. M. Woollcombe. 1951. Resolution of strawberry virus complexes. IV. The latent period of virus 3 in the vector. Ann. Appl. Biol. **38**: 389–94.

Simons, J. N. 1954. Vector-virus relationships of pea-enation mosaic and the pea aphid *Macrosiphum pisi* (Kalt.). Phytopath. **44**: 283–9.

Smith, K. M. 1931. Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leafroll. Ann. Appl. Biol. **18**: 141–56.

Stubbs, L. L. 1948. A new virus disease of carrots: its transmission, host range, and control. Australian Jour. Sci. Res. B **1**: 303–32.

Sylvester, E. S. 1949. Transmission of sugar beet yellow-net virus by the green peach aphid. Phytopath. **39**: 117–32.

Sylvester, E. S. 1956. Beet yellows virus transmission by the green peach aphid. Jour. Econ. Ent. **49**: 789–800.

Insecticide Residues on Vegetables[1]

CHARLES H. BRETT and T. G. BOWERY

ABSTRACT

Snap beans, tomatoes, and collards were dusted at the rate of 30 pounds per acre when they were ready for harvest. Samples were taken immediately after treatment and daily thereafter. All residues showed a linear decrease except for the effect of rainfall. The initial residue on collards was much greater than on snap beans. Snap beans had a greater amount of residue than tomatoes. DDT (10%), methoxychlor (5%), and toxaphene (20%) persisted for at least 12 days on collards and snap beans. There was no detectable toxaphene residue on tomatoes after the ninth day and DDT and methoxychlor residues were nearly gone after the 12th day. Lindane (1%) residues were detectable up to the third day on tomatoes, up to the fourth day on snap beans, and only as a trace on collards by the 13th day. Malathion (4%) and endrin (2%) were detectable for 3 days on snap beans, 2 days on tomatoes, and 3 days on collards. Rainfall removed them from the collards on the fourth day. Rainfall effected a greater reduction of residues during the early period after application than during later periods. Methods used for determining residues are cited.

It is important to know as accurately as possible, the persistence of residues on vegetables, especially for insecticides that are frequently used. Numerous tests have been conducted on different aspects of this problem but generally those studies have not been concerned with residue levels from insecticides applied to vegetables when ready for harvest. Such information would be of value because occasionally it is necessary to control insects during this critical period.

Ashdown & Watkins (1948) found that when applications of DDT were discontinued on lettuce heads 2 to 3 weeks prior to harvest, residues were less than 1 p.p.m. Sloan *et al.* (1951) detected a residue of 1 p.p.m. of DDT on lettuce heads when the application was within 11 days of harvest, and 5.4 p.p.m. when application was within 4 days of harvest. Robinson (1949) applied 5% DDT dust to cabbage heads 4 inches in size. The residue at harvest was 0.07 p.p.m. Wolfenbarger & Van Middelem (1955) indicated that residues of DDT, malathion, and several other materials on greenwrap tomatoes were less than 2 p.p.m. at 0.2 day after final spray application. A semi-logarithmic relationship existed between p.p.m. of residues and time after application. Fahey *et al.* (1956) working with corn, found that DDT emulsions left greater residues on leaves, but granules left greater residues in whorls and leaf axils. Waites & Van Middelem (1955) showed very little difference between the amount of residue from emulsifiable concentrates, wettable powders, or dusts of toxaphene, parathion, and malathion applied to some Florida vegetables.

Westlake & Butler (1953) detected that snap beans with a residue of 0.2 p.p.m. of malathion immediately after application had only a trace after 6 days. Spinach with a residue of 60.9 p.p.m. immediately after application had only 4 p.p.m. after 7 days. Smith *et al.* (1954), in greenhouse tests, found that a malathion residue of 21.2 p.p.m. on mature head lettuce dropped to 0.3 p.p.m. after 9 days. Residues on tomato fruits dropped from 1.05 p.p.m. to 0.2 p.p.m. after 9 days. It was also established by Smith *et al.* (1955) that leafy vegetables had higher residues than snap beans or tomato fruits and that malathion residues could be reduced to low levels by usual washing procedures. Cox *et al.* (1957) showed that malathion sprayed as a wettable powder gave better control of the European corn borer in sweet corn than when sprayed as an emulsifiable concentrate. The emulsifiable concentrate sprays left higher residues but were not so persistent. Granules left considerably less residue but persisted longer than emulsifiable concentrates. All residues were gone after 5 days. Wallis *et al.* (1957) studied malathion residues on eight leafy vegetables and eight fleshy vegetables and noted that in most tests residues on leafy vegetables remained above 8 p.p.m. for 3 days or longer when no rain occurred. In general, the residues on fleshy vegetables were very low 1 hour after treatment.

It was apparent from these experiments that plants with a large surface area in relation to total plant weight had the greatest amount of residue in p.p.m. It was also shown that particle size of carrier materials may affect the initial amount of residue and its duration, *i.e.* larger

[1] Contribution from the Entomology Department and the Chemistry Department, North Carolina Agricultural Experiment Station, Raleigh. Published with the approval of the Director of Research as Paper No. 924 of the Journal Series. Accepted for publication June 3, 1958.

particles effect a lower initial residue with longer duration than small particles, and some insecticides persist much longer than others.

The duration of residues is affected by weather conditions (especially rainfall), plant growth, and many other factors. For this reason tests were conducted in the field under conditions which would minimize losses.

METHODS.—Applications were made at harvest time. Plant material was collected daily and taken into the laboratory for extraction. Temperatures and rainfall were recorded daily. These experiments were conducted during the growing seasons of 1956 and 1957.

Tomatoes were selected because the large fruits offer great volume with little surface area. Collards were selected for their large surface area and relatively small volume. Snap beans were used as an intermediate between tomatoes and collards regarding both surface area and volume.

Insecticide dusts used in the tests were 5% DDT, 10% methoxychlor, 20% toxaphene, 4% malathion, 1% lindane, and 2% endrin. These were applied at the rate of 30 pounds per acre with a single outlet hand duster. Application was made along both sides of the row and on the top.

Two 100 foot rows of each vegetable were treated with each material. In another field, vegetables planted in the same manner were not treated. These were used as checks for correcting the residue analysis. The initial harvest was made immediately after treatment from both treated and untreated plots. Each tomato sample consisted of 15 fruits from a 100 foot row. Two and one-half pounds of snap beans and 20 collard leaves were picked from each 100 foot row. These field samples were delivered daily to the Pesticide Residue Laboratory for subsequent processing and analysis.

The materials received from each of the two field plots were pooled and sub-sampled to give laboratory samples of 500 grams for collards, 1000 grams for beans, and 1500 grams for tomatoes. The samples were then tumble-extracted with Na-distilled n-hexane for DDT, methoxychlor, toxaphene, malathion and endrin, and with carbon tetrachloride (Reagent Grade) for lindane. The ratio of solvent to sample (ml./gm.) was 2, 1, and 0.5 for collards, beans, and tomatoes, respectively. Extraction was continued for 60 minutes at 50 r.p.m. The extracts were filtered and concentrated in Danish-Kuderma concentrators fitted with water cooled Freiderichs condenser heads. The concentrate extracts were transferred to 100-ml. volumetric flasks, made up to volume and stored at 20° C. until analyzed.

At the time of analysis, the volumetric flasks were removed from cold storage and allowed to warm up to 25° C. at which time aliquots of the concentrate equivalent to 50 and 100 grams of collards, 100 grams of beans, and 100 grams of tomatoes were withdrawn for DDT, methoxychlor, toxaphene, endrin, and lindane analysis. Aliquots ranging from 400 to 1200 grams were utilized for malathion analysis.

The following relatively specific methods of analysis as modified for use in our laboratory were utilized in this study: DDT was analyzed using the method of Schechter *et al.* (1945) as modified by Downing *et al.* (1951). The method of Fairing *et al.* (1950) as modified by Doble *et al.* (1950) was used for the determination of methoxychlor. The total organic chlorine method of Agazzi *et al.* (1953) and the colorimetric method of the Hercules Powder Company (1956) was used in determining toxaphene residues. Malathion was analyzed by the method of Norris *et al.* (1954) as modified by Conroy (1957). The Shell Development (1957) phenyl azide colorimetric method was utilized for endrin determination. Lindane residues were analyzed using the method of Schechter *et al.* (1952) as modified by Gehrke *et al.* (1956). The minimum detection levels by these methods, under our laboratory conditions, are shown in tables 1, 2, and 3.

RESULTS AND DISCUSSION.—Insecticide residues as found on snap beans are shown in table 1. No rainfall occurred during the period of this test. Temperatures were moderately high. These data show a linear decrease of residue with time over the range used here.

The residues found on tomatoes are given in table 2.

Table 1.—Insecticide residues on snap beans treated[a] during the harvest period. Faison, N. C. 1956.

Temperature Degrees F. Max./Min.	Rainfall (Inches)	Days After Application	Residues in Parts per Million					
			DDT 5%	Methoxychlor 10%	Toxaphene 20%	Malathion 4%	Endrin 2%	Lindane 1%
80/54	0	0	2.3	2.7	8.10	1.75	0.48	0.35
81/58	0	1	2.7	2.2	7.50	1.08	0.25	0.20
78/59	0	2	2.0	2.3	6.80	0.55	0.25	0.15
81/62	0	3	1.5	1.7	5.64	0.33	nil	0.12
85/59	0	4	1.3	1.6	4.95	nil[b]	nil	nil
88/56	0	5	1.4	1.5	4.95	nil	nil	nil
91/64	0	6	—	—	—	—	—	—
93/67	0	7	1.0	0.9	4.15	nil	nil	nil
89/67	0	8	0.8	0.8	3.90	nil	nil	nil
92/64	0	9	0.6	0.7	3.75	nil	nil	nil
93/69	0	10	1.0	0.8	2.18	nil	nil	nil
92/69	0	11	—	—	—	—	—	—
94/66	0	12	0.4	0.7	1.52	nil	nil	nil
FDA tolerance			7.0	14.0	7.0	8.0	—	10.0
Minimum detection level			0.1	0.1	0.15	0.12	0.10	0.10

[a] Dusts were applied at the rate of 30 pounds per acre.
[b] Below detectable levels.

Table 2.—Insecticide residues on tomatoes treated[a] during the harvest period. Faison, N. C. 1956.

Temperature Degrees F. Max./Min.	Rainfall (Inches)	Days After Application	Residues in Parts per Million					
			DDT 5%	Methoxychlor 10%	Toxaphene 20%	Malathion 4%	Endrin 2%	Lindane 1%
82/62	0	0	0.59	1.53	4.00	0.31	0.31	0.28
91/65	0	1	0.61	1.81	3.20	0.25	nil[b]	0.25
96/71	0	2	0.48	1.29	2.77	0.09	nil	nil
98/73	0	3	0.49	0.8	3.00	nil	nil	nil
95/70	0.34	4	0.26	0.57	1.00	nil	nil	nil
95/69	1.18	5	—	—	—	—	—	—
94/67	0	6	0.30	0.27	0.50	nil	nil	nil
94/74	0	7	0.27	0.18	0.60	nil	nil	nil
91/69	1.2	8	0.20	nil	0.30	nil	nil	nil
90/73	0	9	0.19	0.16	0.36	nil	nil	nil
86/67	0	10	0.15	nil	0.15	nil	nil	nil
91/69	0	11	—	—	—	—	—	—
95/69	0	12	0.14	nil	0.15	nil	nil	nil
FDA tolerance			7.0	14.0	7.0	8.0	Zero	10.0
Minimum detection level			0.10	0.10	0.15	0.06	0.20	0.10

[a] Dusts were applied at the rate of 30 pounds per acre.
[b] Below detectable levels.

The initial residue and duration for all insecticides was less on tomatoes than on snap beans or collards. The initial residue for DDT was 0.59 p.p.m. The results of the field test report in table 2 show a total of 1.52 inches of rainfall on the fourth and fifth days after treatment. This appeared to have some effect on residue decrease. Maximum temperatures were generally above 90° F. during the test period.

Residues found on collards are shown in table 3. On the fourth day after application, the amount of residue was considerably decreased by 0.41 inch of rainfall. Subsequent rainfall of 0.6 inch on the seventh day and 0.9 inch on the 11th day had very little effect on the residue remaining.

Five per cent DDT, 10% methoxychlor, and 20% toxaphene persisted for at least 12 days on collards and snap beans. On tomatoes there was no detectable toxaphene residue after the ninth day, and DDT as well as methoxychlor residues were nearly gone by the 12th day. The persistence of toxaphene on collards was probably due to the high initial residue of 168 p.p.m.

One per cent lindane residues were detectable up to the third day on tomatoes and up to the fourth day on snap beans. Lindane was still detectable, but only as a trace, on collards by the 12th day.

Four per cent malathion and 2% endrin were detectable for 3 days on snap beans. They were both detectable only to the second day after application on tomatoes. Residues of these insecticides on collards were present for 3 days, but 0.41 inch of rainfall on the fourth day reduced them below the level of detection.

Two additional tests were made in 1957 to measure malathion residues on tomatoes and snap beans. In the first test the initial residue was 1.11 p.p.m. on snap beans and 0.06 p.p.m. on tomatoes. On the day following application, there was a 0.64 inch rain, and an additional 0.11

Table 3.—Insecticide residues on collards treated[a] during the harvest period. Faison, N. C. 1957.

Temperature Degrees F. Max./Min.	Rainfall (Inches)	Days After Application	Residues in Parts per Million					
			DDT 5%	Methoxychlor 10%	Toxaphene 20%	Malathion 4%	Endrin 2%	Lindane 1%
64/37	0	0	63.0	63.0	168.0	35.8	17.3	7.77
64/36	0	1	43.5	35.0	155.0	23.5	15.2	3.77
63/42	0	2	23.2	31.2	110.0	19.2	3.4	3.47
69/40	0	3	21.7	23.2	106.0	6.5	2.7	2.57
80/60	0.41	4	3.6	3.1	12.9	nil[b]	nil	0.73
80/53	0	5	2.8	2.9	9.3	nil	nil	0.47
74/47	0	6	2.4	2.7	8.1	nil	nil	0.40
75/62	0.6	7	1.8	2.2	7.1	nil	nil	0.32
65/52	0	8	1.4	1.7	6.8	nil	nil	0.27
80/43	0	9	1.2	1.1	6.2	nil	nil	0.26
80/56	0	10	0.9	1.1	5.2	nil	nil	0.24
78/42	0.9	11	1.3	0.8	5.1	nil	nil	0.22
68/35	0	12	0.7	0.5	5.0	nil	nil	0.10
74/40	0	13	0.6	0.4	4.9	nil	nil	nil
FDA tolerance			7.0	14.0	—	—	—	10.0
Minimum detection level			0.10	0.10	1.0	0.25	0.10	0.10

[a] Dusts were applied at the rate of 30 pounds per acre.
[b] Below detectable levels.

on the second day; these rains reduced the residues below the detectable levels. In the second test the initial residue was 1.17 p.p.m. on snap beans and 0.06 p.p.m. on tomatoes. Without rainfall, these residues were detectable for 3 days but could not be measured on the fourth day.

It appears the rainfall has a greater effect on the reduction of residues during the early part of the period than during the latter part. When the surface area of plant material treated is proportionally large, as compared to volume, residues for a given rate of application are greater and persist longer. All of the insecticides in these tests disappeared from the plants at an almost linear rate except for the influence of rainfall.

As of January 1958 the Food and Drug Administration (FDA) has established tolerances of 10 p.p.m. for lindane, 7 p.p.m. for DDT, and 14 p.p.m. for methoxychlor on snap beans, tomatoes, and collards. Tolerances of toxaphene on snap beans and tomatoes are 7 p.p.m. Tomatoes and beans have a tolerance of 8 p.p.m. of malathion. Zero tolerance has been assigned for endrin on tomatoes. All of the initial residues in these tests were considerably below FDA tolerance levels except toxaphene on snap beans which was initially 8.1 p.p.m., but had dropped to 6.8 p.p.m. by the second day, whereas DDT and methoxychlor on collards with initial residues of 63 p.p.m. had dropped below the FDA tolerance levels following 0.41 of an inch of rainfall on the fourth day. Tolerances have not been established on collards for endrin, malathion, or toxaphene, and no tolerance has been established for endrin on beans.

REFERENCES CITED

Ashdown, Donald, and T. C. Watkins. 1948. Control of lettuce yellows disease in New York. Jour. Econ. Ent. 41(2): 252–8.

Agazzi, E. J., E. D. Peters, and F. R. Brooks. 1953. Combustion techniques for the determination of residues of highly chlorinated pesticides by total chlorine analysis. Analyt. Chem. **25:** 237–40.

Anonymous. 1955. Photometric determination of endrin in agricultural crops—dechlorination-phenyl azide sulfanilic acid method. Shell Development Company. ARMS-C-10/55.

Anonymous. 1956. Colorimetric method for toxaphene. Hercules Powder Company, IM No. 63.

Conroy, H. W. 1957. Report on malathion. Jour. Assoc. Offic. Agric. Chemists **40**(1): 230–5.

Cox, H. C., J. E. Fahey, and T. A. Brindley. 1957. European corn borer control in relation to malathion residues. Jour. Econ. Ent. **50**(1): 52–5.

Doble, J., and W. Thornburg. 1950. A modified procedure for the determination of methoxychlor. FDA tolerance hearings, Exhibit 1247-A.

Downing, G., and L. B. Norton. 1951. Modification of Schechter method of estimating DDT residue. Analyt. Chem. **23:** 1870.

Fahey, Jack E., H. W. Rusk, and H. C. Cox. 1956. Residues on plants treated with DDT granules and emulsions for European corn borer control. Jour. Econ. Ent. **49**(6): 846–9.

Fairing, J. D., and H. P. Warrington, Jr. 1950. Colorimetric determination of small quantities of 1,1,1-trichloro-2,2-bis(P-methoxyphenyl)-ethane. Adv. Chem. Ser. No. **1:** 260–5.

Gehrke, C. W., and J. L. Bevirt. 1956. Critical points in the Schechter-Hornstein colorimetric method. University of Missouri Res. Bull. **606.**

Norris, M. V., W. A. Vail, and P. R. Averell. 1954. Colorimetric estimation of malathion residues. Jour. Agric. and Food Chem. **2:** 570–3.

Robinson, R. H. 1949. Spray residues and precautions relative to handling new spray materials. Oregon State Hort. Soc. Proc. **41:** 34–7.

Schechter, M. S., and I. Hornstein. 1952. Colorimetric determination of benzene hexachloride. Analyt. Chem. **24:** 544–8.

Schechter, M. S., S. B. Soloway, R. A. Hayes, and H. L. Haller. 1954. Colorimetric determination of DDT. Indust. and Engineer, Chem., Analyt. Ed. **17:** 704–8.

Schiffman, C. D. 1957. Report on methoxychlor residues on fruits and vegetables. Jour. Assoc. Offic. Agric. Chemists **40**(1): 235–8.

Sloan, M. J, W. A. Rawlins, and L. B. Norton. 1951. Residue studies on DDT and parathion applied to lettuce for control of the six-spotted leafhopper. Jour. Econ. Ent. **44**(5): 691–701.

Smith, Floyd F., Paul Giang, and R. A. Fulton. 1954. Residues of malathion on greenhouse lettuce and tomatoes and on green onions. Jour. Econ. Ent. **47**(1): 183–4.

Smith, Floyd F., Paul Giang, and Edgar A. Taylor. 1955. Reduction of malathion residues on vegetables by washing. Jour. Econ. Ent. **48**(2): 209–14.

Waites, Robert E., and C. H. Van Middelem. 1955. Residue studies of toxaphene, parathion, and malathion on some Florida vegetables. Jour. Econ. Ent. **48**(5): 590–3.

Wallis, R. J., Floyd F. Smith, Helen G. Wheeler, and Edgar A. Taylor. 1957. Malathion residues on vegetable, berry, and tobacco crops. Jour. Econ. Ent. **50**(3): 362–3.

Westlake, William E., and Lillian I. Butler. 1953. Residues of malathion on fruits and vegetables. Jour. Econ. Ent. **46**(5): 850–2.

Wolfenbarger, D. C., and C. H. Van Middelem. 1955. Reduction of insecticidal residue on mature green-wrap tomatoes. Jour. Econ. Ent. **48**(6): 744–6.

ERRATUM

In table 2 on page 723 of Vol. 51, No. 5, October, 1958, "Per Cent Reduction" should be deleted from the heading over the last three columns. The figures given are for actual populations instead of percentages.

Corn Earworm Control in Sweet Corn[1]

W. G. Eden, *Department of Zoology-Entomology, Alabama Polytechnic Institute, Auburn*

Abstract

Experiments on control of the corn earworm, *Heliothis zea* (Boddie), in sweet corn were conducted in Alabama in 1956 and 1957. Daily applications of 2.0 pounds per acre of DDT as an emulsion spray during the pre-silk and silking periods resulted in over 99% worm-free ears. Results of the experiments showed that the early part of the silking period was the most critical time for protecting corn against the corn earworm. DDT at 2.5 pounds per acre per application was superior to Phosdrin at 0.25 pound and Chlorthion at 1.0 pound. Thiodan at 2.0 pounds compared favorably with DDT at 2.0 pounds. DDT at 1.0 pound was superior in effectiveness to toxaphene at 2.0 pounds, Hercules AC-528 at 0.5 pound, heptachlor at 0.5 pound, malathion at 0.5 pound, and parathion at 0.25 pound. Sevin (1-naphthyl-N-methyl carbamate) at 2.0 pounds compared favorably with DDT but the formulation used caused severe foliage injury.

Control of the corn earworm, *Heliothis zea* (Boddie), is an essential operation in the production of marketable sweet corn in Alabama. Research on control of this insect has been conducted for several years in Alabama and other states where sweet corn is grown. This research has established that DDT is a good insecticide for earworm control. Results of experiments on intervals and timing of DDT sprays in Alabama were reported in 1956 (Eden 1956).

Materials and Procedure.—The two experiments reported on herein were conducted at the Gulf Coast Substation of the Agricultural Experiment Station of the Alabama Polytechnic Institute. This substation is located near Fairhope, Alabama, on the eastern shore of Mobile Bay. One experiment was conducted in 1956 and one in 1957.

The variety of sweet corn used in the experiments was Golden Security. Each plot consisted of four rows of corn from 70 to 100 feet long. The treatments in both experiments were replicated four times in randomized blocks. All spray formulations were emulsions. The sprays were applied with a high-clearance, self-propelled power sprayer equipped with a fixed boom and four flat fan nozzles (No. 65067) per row. The nozzles, two on each side of the row, were attached to drops from the horizontal boom and directed spray into the ear region of the stalks. The sprays were applied at 125 to 150 pounds pressure and at 20 to 25 gallons per acre.

The 1956 experiment was designed to determine the influence of interval between DDT sprays when the same interval was maintained throughout the protection period and when the interval was varied in either the first or the latter portions of the spray period. This experiment also included a series of treatments to determine: the influence of varying the time of beginning earworm spray schedules; the effect of reversing the direction of the spraying machine with alternate applications; and to compare the effectiveness of DDT-mineral oil, Phosdrin, Thiodan, and Chlorthion with DDT spray for earworm control. In the 1956 experiment, some sprays were applied at an indicated number of days before silking. These dates were determined by cutting ear shoots and observing the rate of silk development. In all cases, the stalk was indicated as being in silk as soon as the first silks began to show through the tip of the husk.

The experiment in 1957 included further studies on intervals between sprays, times of beginning spray programs, and comparisons of DDT with several other insecticides for earworm control. Spraying was begun on some plots when the stalks were shooting freely or when about 80% of the stalks were showing ear shoots.

Results of both experiments were determined by examining the primary ears for earworm damage when the corn was ready to harvest. One hundred ears taken consecutively on the two inside rows of each plot were examined.

Results.—Results of the 1956 experiment are presented in table 1. The highest percentage of worm-free ears, 96.5, resulted from 15 daily applications of 2.5 pounds of DDT starting the first day after the first silks appeared. This treatment was significantly more effective than the 2- or 3-day interval schedules, which required 8 and 5 applications and resulted in 84.5 and 65.5% worm-free ears, respectively. The 15-application 1-day interval spray schedule, however, did not result in significantly more worm-free ears than schedules of 1-day intervals for 7 days followed by sprays applied at intervals of 2 or 3 days for the remainder of the protection period. These treatments resulted in 95.5 and 91.8% worm-free ears, respectively. The 15-application 1-day interval schedule and the schedules of seven 1-day interval sprays followed by sprays at intervals of 2 or 3 days were more effective than schedules of 2- or 3-day intervals for the first half of the protection period followed by sprays at 1-day intervals.

In the series of schedules designed to determine the most effective date to begin spraying, there were no significant differences in the percentages of worm-free ears resulting from starting 3 days or 1 day before silking or 1 day after silking began. However, the percentages of worm-free ears did decrease as the starting date was delayed, and this trend was significant. Reversing the direction of the spray machine with alternate applications in this series had no effect on the earworm control obtained.

DDT at 1.5 pounds with 1.75 gallons of mineral oil per acre per application sprayed at 2-day intervals resulted in 91.0% worm-free ears as compared with 84.5% for 2.5 pounds of DDT without oil. The difference was not significant at the 5% level of probability. Thiodan at 2.0 pounds per acre per application compared favorably with 2.5 pounds of DDT. Phosdrin and Chlorthion were ineffective at the rates used.

Results of the 1957 experiment are presented in table

[1] Accepted for publication June 5, 1958

Table 1.—Worm-free ears in sweet corn following various insecticidal treatments. Fairhope, Alabama. 1956.

Treatment[a]	No. Applications	Worm-Free Ears	
		Per Cent	Angle
Untreated check	0	1.0	4.90
DDT, 2.5 lb./A./application; began spraying 1 day after first silks appeared; intervals as indicated			
1-day	15	96.5	81.69
2-day	8	84.5	67.28
3-day	5	65.5	54.07
1-day for 7 applications; 2-day thereafter	11	95.5	78.37
2-day for 4 applications; 1-day thereafter	11	81.8	65.05
3-day for 3 applications; 1-day thereafter	9	52.5	46.44
1-day for 7 applications; 3-day thereafter	9	91.8	74.07
DDT, 2.5 lb./A./application, applied at 2-day intervals until 1 spray was applied to full silk; 3-day intervals thereafter; spray schedules started as follows:			
3 days before silking began	9	89.8	71.73
1 day before silking began	8	84.0	67.00
1 day after silking began	7	81.0	64.43
1 day after silking began; reversed directions of machinery with alternate applications	7	80.0	63.61
DDT, 1.5 lb. and 1.75 gal. mineral oil/A./application; began spraying 1 day after first silks appeared; 2-day intervals throughout	8	91.0	74.20
Began spraying 1 day after first silks appeared; 2-day intervals until 1 spray was applied to full silk; 3-day intervals thereafter			
Phosdrin, 0.25 lb./A./application	7	8.8	16.63
Thiodan, 2.0 lb./A./application	7	81.3	64.75
Chlorthion, 1.0 lb./A./application	7	12.3	20.34
L.S.D. at 5% level			8.54
at 1% level			11.41

[a] Spraying was continued on all plots until the silks turned brown. Part of the experiment was involved in a nationally coordinated experiment.

Table 2.—Worm-free ears in sweet corn following various insecticidal sprays. Fairhope, Alabama. 1957.

Treatment[a]	No. Applications	Worm-Free Ears	
		Per Cent	Angle
Untreated check	0	6.8	14.88
DDT, 2.0 lb./A./application; began spraying when stalks were shooting freely; intervals as indicated			
1-day	21	99.5	87.13
2-day	11	96.3	80.34
3-day	8	86.3	68.26
3-day until silking began; 1-day until full silk; 3-day thereafter	12	98.3	83.65
Same as above less first spray	11	99.3	86.53
Same as above less first and second sprays	10	98.5	84.04
DDT, 2.0 lb./A./application; began spraying the day first silks appeared; 1-day intervals until 1 spray was applied to full silk; 3-day intervals thereafter	10	98.6	79.24
Began spraying when stalks were shooting freely; 3-day intervals until silking began; 1-day intervals until 1 spray was applied to full silk; 3-day intervals thereafter; insecticide rate in lb./A./application			
DDT, 1.0	12	95.5	78.62
Toxaphene, 2.0	12	32.5	34.74
Endrin, 0.25	12	74.5	59.79
Hercules AC-528, 0.5	12	55.0	47.88
Sevin, 2.0	12	96.5	79.51
Heptachlor, 0.5	12	60.0	50.94
Malathion, 0.5	12	51.0	45.53
Parathion, 0.25	12	58.0	49.64
L.S.D. at 5% level			6.06
at 1% level			8.10

[a] Spraying was continued on all plots until the silks turned brown.

2. Twenty-one daily sprays of 2 pounds of DDT beginning when the stalks were shooting freely resulted in 99.5% worm-free ears as compared with only 6.8% in the unsprayed corn. This program resulted in significantly more worm-free ears than the 2-day interval spray schedule (96.3%). The 2-day interval schedule resulted in significantly more worm-free ears than that obtained by sprays applied at 3-day intervals (86.3%). The 1-day interval schedule did not result in significantly better worm control than a schedule consisting of 3-day intervals until silking began, 1-day intervals until full silk, and followed by sprays at 3-day intervals (98.3% worm-free ears). Furthermore, leaving off the first and/or the first and second sprays of the latter schedule did not reduce the percentages of worm-free ears.

DDT at 1 pound per acre resulted in almost as good control as 2 pounds when 12 applications were made, seven of which were applied at 1-day intervals during the silking period. Sevin (1-naphthyl-N-methyl carbamate) at 2.0 pounds per acre per application compared favorably with 2.0 pounds of DDT; however, the formulation caused severe foliage injury. Toxaphene, endrin, Hercules AC-528, heptachlor, malathion, and parathion at the rates used were all much less effective than 1.0 pound of DDT.

Reference Cited

Eden, W. G. 1956. Experiments on earworm control in sweet corn. Jour. Econ. Ent. **49**(6): 822–5.

Feeding Behavior and DDT Resistance of *Epitrix cucumeris* (Harris)[1]

JAMES B. KRING,[2] *The Connecticut Agricultural Experiment Station, New Haven*

Abstract

In laboratory tests resistant potato flea beetles (*Epitrix cucumeris* (Harris)) fed more on DDT-treated potato foliage, with less mortality, than the susceptible beetles. Both resistant and susceptible beetles preferred untreated to DDT-treated foliage. This preference was greater for susceptible beetles than it was for resistant ones. A positive response in feeding orientation was demonstrated for light, physical injury to the feeding surface, and increase in temperature. A negative response away from DDT, dieldrin and malathion was also demonstrated. Negative response to DDT was less where light and physical leaflet surface were used as antagonistic stimuli.

The behavior of the potato flea beetle, *Epitrix cucumeris* (Harris) was used to investigate DDT resistance in this insect. Since Bordeaux mixture and several other compounds are repellent to flea beetles (Jones 1894, Turner 1940), it seemed reasonable to consider that repellency might be a part of the action of DDT. Effects of DDT on behavior of certain other insects have already been demonstrated and the subject has been reviewed by Dethier (1956).

Failure of DDT against the potato flea beetle was first observed in 1951 (Gould & McCrosky 1954, Kring 1955, 1956). At that time only five or six generations of flea beetles had been exposed to this chemical. Resistance to DDT is now reported from potato growing areas in Wis-

[1] Accepted for publication June 9, 1958.
[2] The technical assistance of Mr. A. DeCaprio is gratefully acknowledged.

Table 1.—Laboratory tests showing the effect of DDT on mortality and feeding rate of potato flea beetles.[a]

DDT	Resistant		Susceptible	
	Per Cent Mortality	Feeding Punctures per Survivor	Per Cent Mortality	Feeding Punctures per Survivor
1- 800	24	3.37	48	0.08
1-1600	16	5.86	24	1.84
1-3200	12	11.73	16	7.90
None	—	9.82	—	12.55

[a] Two treated or two untreated potato leaflets in each replicate. 1953.

consin (Fisher 1957) Virginia (Hofmaster 1956), Rhode Island (Kerr 1957), and Ohio (Sleesman 1955).

PROCEDURE.—The insects used were swept from selected areas and stored at 45±2° F. A 4″×4″×3″ clear plastic box with a tight fitting soft plastic cover was used for storage. Ventilation was provided by making a large number of small holes in the cover. High mortality was observed in storage with condensation and decay of organic matter. To avoid this, beetles were stored without food. Some moisture was necessary and this was provided on pieces of moist cotton.

Before testing, flea beetles were moved from the storage temperature to a higher temperature (70±2° F.). The box cover was replaced by another inverted box of the same size. Only beetles migrating from the lower to the upper box were used. It was possible to transfer known numbers of insects easily by first anesthetizing the beetles with CO_2 and then storing them briefly in corked test tubes.

The studies of feeding behavior and effect of insecticides were made on beetles isolated in 60-mm. glass petri dishes or 70-mm. plastic petri dishes. One or two excised potato leaflets and a small, moistened plug of cotton were placed in each chamber. Five or 10 insects were then added to each dish.

To test the reaction of this insect to DDT and other insecticides, leaflets were treated by spraying one surface with a test insecticide. Applications were made using a laboratory sprayer described by Turner & Woodruff (1946). The leaflets were allowed to dry before placing them in the test chambers.

The DDT formulation used in these tests was a 25% emulsion concentrate prepared with xylene solvent (60%) and Triton X-100 emulsifier (15%). A 12% dieldrin emulsion concentrate was also used, prepared with xylene solvent (84.5%) and Triton X-155 (1.5%) and Triton B-1956 (2%) emulsifiers.

Feeding activity was measured by counting the feeding punctures in each surface of the potato leaflets.

In one series of tests flea beetles were dipped in DDT. In such tests the beetles were shaken in an emulsion of 1 part DDT to 800 parts of water for 1 minute, removed, dried and placed in a clean container with uncontaminated food.

Except as indicated, feeding tests were conducted in the absence of light to avoid the influence of any beetle response to light on the results. All tests were replicated three to five times.

RESULTS.—*Reaction to DDT.*—In 1953, the effectiveness of DDT against the potato flea beetle varied greatly from one location to another (Kring 1955). This variation appeared to be related to the amount and frequency of the use of DDT in prior years.

Potato flea beetles collected from locations where DDT was and was not effective were compared. Insects from both groups were fed on potato leaflets treated with different dosages of DDT. By recording the mortality and feeding rate at intervals of time after the flea beetles were introduced into the test, it was possible to demonstrate both mortality and feeding differences between groups of insects collected from these two types of areas (table 1). It was shown that the susceptible beetles were more easily killed, and the survivors fed less, than the resistant beetles.

This difference indicated that perception of DDT might be involved. Although most of the surviving beetles were not visibly affected by the DDT, the number of feeding punctures per surviving beetle in both groups was reduced as the amount of DDT was increased.

Detection of DDT.—Tests in 1954 suggested that potato flea beetles could detect DDT. Beetles given a choice between treated and untreated potato leaflets fed on the untreated leaflets (fig. 1). Preference for an untreated leaflet over a treated leaflet decreased as the amount of toxicant was reduced (table 2).

When susceptible and resistant beetles were compared (table 2) a marked difference was noted. Both ate less of the treated than of the untreated leaflets. However, this preference for untreated leaflets was greater for susceptible beetles than it was for resistant ones. Resistant beetles ate two to six times more of treated leaflets and there was also a lower mortality.

Since contact with DDT was not always assured in feeding tests, flea beetles were dipped in an emulsion of 1 part DDT to 800 parts of water. This was done to determine if resistance was simply the avoidance of DDT. The mortality of flea beetles dipped in the same dilution of solvent and emulsifier (16.5%) without DDT was nearly equal to that with the DDT emulsion (23.1%) itself. Both solutions reduced the feeding of surviving beetles about 50% below that of a treatment with water alone.

To determine the mortality trends and the differences

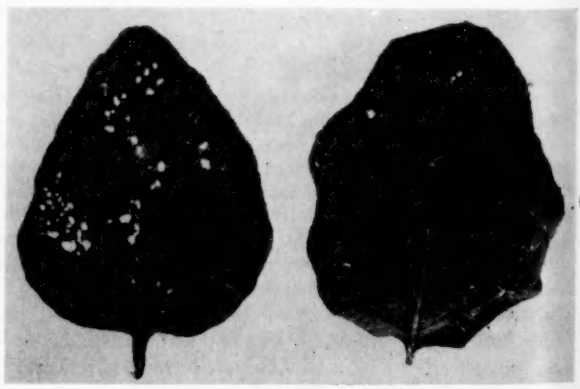


FIG. 1.—Feeding preference of potato flea beetles for untreated potato leaflets (left) in the dark.

in the ability of the potato flea beetle to detect DDT, similar tests were made on beetles from a single area for three successive years—1954, 1955, and 1956. Figure 2 graphically illustrates the comparisons of the reaction of beetles collected from this area in each of these 3 years. Both mortality and amount of feeding decreased. While the number of bites in treated surfaces was relatively high in some of these tests, the surface did not appear heavily damaged because the bites were smaller and shallower than usual.

Detection of Other Insecticides.—Turner (1934, 1940) demonstrated in the laboratory that Bordeaux mixture repelled but did not kill potato flea beetles.

To determine the extent of this ability to detect insecticides, dieldrin and malathion were tested. Dieldrin is effective against the potato flea beetle whereas malathion is not. Dieldrin at concentrations as low as 1 to 80,000 was detected. At half this concentration the detection was very slight (table 2). When the concentration was greater than 1 to 20,000 the mortality after 24 hours was so high that it was not possible to determine if any detection occurred before death. Malathion, however, was detected at 1 to 800 concentration, 88.9% of the total bites being in the untreated leaflets, and there was no mortality.

Leaflets sprayed with a test solution of 1 part xylol and Triton X-100 to 800 parts of water were not avoided. When DDT was omitted from the spray solution treated leaflets received as many feeding injuries as untreated leaflets.

Both DDT and dieldrin caused tremors. With dieldrin, and in the early DDT tests, these tremors were usually followed by death. Flea beetles poisoned by dieldrin also excreted clear body fluids which often hardened on the posterior of the beetle. When large amounts were excreted, the beetles stuck to the sides of the test container and died in this position. The nature of this material is not known.

Table 2.—Feeding preference response of potato flea beetles to DDT and dieldrin in laboratory tests.

Insecticide and Dilution	No. of Bites[a]		Per Cent Difference	In 50 Beetles		Bites per Survivor
	Treated Leaflets	Untreated Leaflets		Dead	Tremors	
DDT susceptible beetles collected Mt. Carmel, 1954						
DDT						
1- 800	141.5	—	—	27	3	12.3
1- 800	21	285	92.6	14	4	8.5
1-1600	66	749	91.5	10	4	20.3
1-3200	109	908	88.0	7	1	23.6
1-6400	290	1147	74.7	1	2	29.3
None	—	1212.5	—	0	0	48.6
DDT resistant beetles collected Windsor, 1954						
DDT						
1- 800	184.5	—	—	11	4	9.5
1- 800	118	961	87.7	9	1	26.3
1-1600	412	1019	59.5	6	1	32.5
1-3200	419	823	49.0	6	0	28.2
1-6400	730	989	26.1	4	0	37.3
None	—	981.5	—	1	0	39.2
DDT resistant beetles collected Somers, 1956						
DDT						
1- 800	168	1038	86.1	0	0	24.1
1-1600	425	900	67.9	0	0	26.5
1-3200	681	923	57.5	0	0	32.1
1-6400	601	1109	64.9	0	0	34.2
Dieldrin						
1- 20,000	145	455	68.1	34	15	37.5
1- 40,000	187	359	47.9	20	29	18.2
1- 80,000	384	666	42.3	19	27	33.9
1-160,000	719	745	3.5	1	30	29.9
None	—	990.5	—	0	0	39.6

[a] Two potato leaflets in each replicate; both treated, one treated, or both untreated.

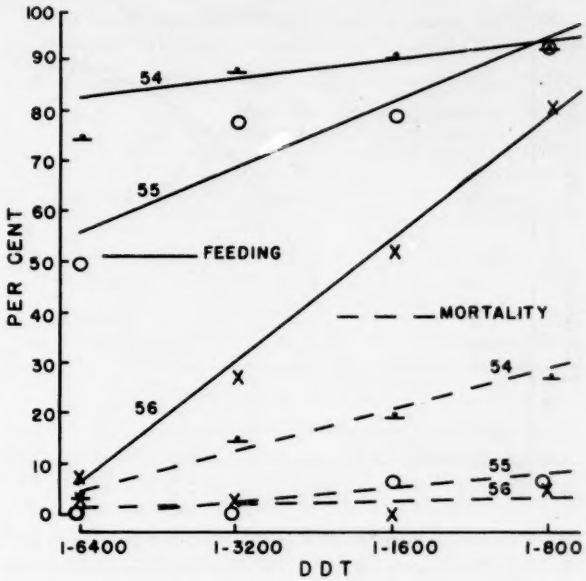


FIG. 2.—Decrease in mortality (dotted lines) and in feeding preference (solid lines) of potato flea beetles for untreated potato leaflets (over a 3-year period).

In other laboratory tests when resistant flea beetles were fed DDT, some of them developed tremors similar to those in DDT poisoning. Within 48 hours, these tremors disappeared and the beetles behaved normally.

Mechanics of Potato Flea Beetle Feeding.—Better understanding of the results of the preceding tests was obtained by recording the feeding behavior of the potato flea beetle. The beetles were frequently observed to clean their legs and tarsi. Mouth parts were exercised vigorously during and following this activity. The flea beetles tested the leaf surface with the tarsi of the first pair of legs before feeding. This initial probing was followed by an examination of the leaflet surface with the maxillary palps and to a lesser extent with the labial palps. Once a feeding site was selected, penetration of the surface was made with the mandibles. In active feeding the legs were moved and braced to exert maximum body power. Tissues were pulled and torn from the leaflet. A single hole made by feeding did not usually represent the feeding activity of a single beetle but rather the passing attack of several flea beetles.

Effect of Temperature on Feeding.—In periods of early cold weather (nights with light frost) potato flea beetles were observed to have fed extensively on the underside of leaflets over 10 inches above the soil. In contrast to this it was also observed that within 4 inches of the surface of the soil most feeding was on the upper leaflet surface.

This observation suggested that temperature extremes can play an important part in the selection of either the upper or lower surface of the leaflet as a feeding site. In the field in July and August there was no difference in the choice of feeding surface between upper and lower or inner and outer leaflets. For this reason, it was assumed

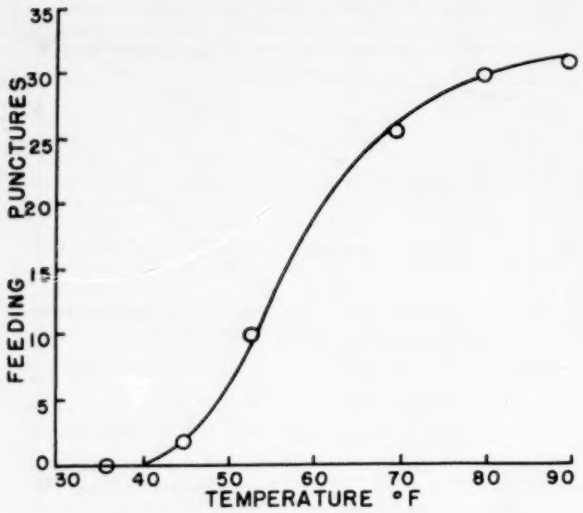


FIG. 3.—Average number of feeding punctures per potato flea beetle at six constant temperatures, 24-hour test, in the dark.

that only extreme temperature conditions affect the choice of surface.

The effect of constant temperatures of 36, 45, 53, 70, 80 and 90° F. on the feeding rate was investigated (fig. 3). Here feeding rate was expressed in the number of bites per surviving beetle in a 24-hour period. In this test, feeding was initiated at 45° F. and the rate increased rapidly to the temperature of 70° F. There was little increase in rate above 70° F. However, in earlier tests at this temperature the average number of feeding punctures per surviving beetle varied greatly. This extreme variation was due to starvation. By feeding the beetles before testing, variability between tests was reduced.

Attractiveness of Injured Leaflet Tissues.—It was observed that feeding punctures were grouped, rather than evenly distributed over the leaflet surface (fig. 1). Flea beetles, given a choice of leaflets previously fed on by other beetles and undamaged leaflets stored under the same conditions, congregated on the damaged leaflets. As the undamaged leaflet was fed upon, and as the amount of suitable feeding area decreased on the damaged leaflet, the beetles began to feed on both types of leaflets in equal numbers. Within 280 minutes the distribution of feeding beetles on the leaflets was reversed from the distribution which occurred within 40 minutes after the start of the test.

By substituting mechanical damage for the flea beetle feeding it was possible to show that detection occurs when the amount of damaged surface was between 4 and 8% (table 3).

Spraying the leaflets with the washings from storage boxes in which beetles had been maintained did not make them more attractive, *i.e.*, no response similar to the "fly factor" of Barnhart & Chadwick (1953).

Effect of light.—In the dark and under florescent lights (150 and 300 foot candles) potato flea beetles fed more on the upper surface of potato leaflets than on the lower. This preference increased with the intensity of the light (table 4).

When potato flea beetles were given a choice between two leaflets, one slightly nearer a source of light than the other, the most feeding occurred on the leaflets near the light.

Effect of leaflet surface.—Various workers have indicated that although potato flea beetles fed on both upper and lower leaflet surfaces, most feeding was on the lower surface of the leaflet. Examination of leaflets from untreated field-grown plants in Connecticut showed that the beetles had fed on both surfaces of the leaflets. When random samples were taken and the total number of feeding punctures for each surface were compared, it was found that the upper surface was more heavily fed upon than the lower. In Kathadin potatoes as high as 95% of the total number of feeding punctures were in the upper surface. In midsea on the number of feeding punctures was higher in the upper surface of the leaflets than in the lower whether the sample was taken from the upper, middle or lower vertical position on the plant. However, in one field of another variety of potatoes, Irish Cobbler, the upper surface was only slightly favored over the lower surface.

Since the actual surface of the leaflet rather than its position in space might effect the choice of feeding surface, leaflets from both Kathadin and Irish Cobbler potato plants were compared in the laboratory. Using leaflets of these two potato varieties in small plastic petri dishes and exposing the upper leaflet surface in one series and the lower in another, it was possible to evaluate the preference of the potato flea beetle for a particular surface of the leaflet in the dark (table 5). In all tests the lower surface of the leaflet was not as extensively damaged as the upper. The beetles did feed more extensively on the surface not in contact with the floor of the test chamber. Inverting the leaflet did not completely overcome the feeding preference for the upper surface of the leaflet in either variety.

Leaflet surface vs. light.—Since light and leaflet surface both affect the location of feeding punctures on the leaflet, and since both of these factors could affect beetle activity in the field, they were compared. This was easily done by adding light as a stimulus in the preceeding experiment discussed above. The amount of feeding was increased in the lower surface of the inverted leaflet (table 5). This was true of both varieties. Light exerted the stronger influence.

Light and leaflet surface response vs. DDT.—Positive reaction to light and upper surface was demonstrated. DDT was also shown, in tests where light did not influence the results, to repel flea beetles. By conducting preference tests where light was used as an additional

Table 3.—Effect of amount of artificial injury on attractiveness of individual potato leaflets to potato flea beetles.

PER CENT LEAFLET SURFACE INJURED	PER CENT BEETLES FEEDING ON	
	Injured Leaflet	Uninjured Leaflet
4	48.8	51.2
8	75.0	25.0
16	82.8	17.1
32	97.9	2.1
64	88.6	11.4

stimulus it was possible, partially, to overcome the DDT effect. This was done by using light to attract the beetles to the DDT treated leaflet. A more complete reaction away from DDT could be obtained by using both the repellent action of DDT to drive the beetles from the treated leaflet and light to attract them to the untreated leaflet. In the absence of light the same beetles preferred untreated to DDT-treated leaflets.

The same response also could be shown where use was made of the positive response of the beetles to the upper surface of the leaflet. When light was used as the stimulus, a DDT treatment of the leaflet surface exposed to the light only slightly reduced the preference for that surface. In the dark, with the upper leaflet surface exposed, 76.9% of the total feeding punctures were in the upper surface. In light, this percentage was increased to 81.7%. Spraying of this surface with DDT emulsion at 1 to 800 dilution reduced this percentage to 66.3%.

DISCUSSION.—The ability of the potato flea beetle to detect DDT is not an example of behavioristic resistance as discussed by Grayson & Cochran (1955) because both susceptible and resistant flea beetles could detect DDT. Furthermore, DDT-resistant beetles fed more on treated foliage than susceptible beetles. As the susceptible population became more resistant, perception of DDT, as indicated by these tests, decreased.

Detection and avoidance of DDT as demonstrated here has probably influenced the rate of development of resistance. Such reactions could permit longer survival of beetles tolerant to DDT by exposing them only to sublethal amounts of DDT.

It was also shown that this insect could detect a number of other chemicals. This ability might permit the potato flea beetle to develop strains resistant to other insecticides substituted for DDT.

In one locality during a 3-year period there was a progressive reduction in the detection of DDT. This may be correlated with a reduction in mortality. However, the level of DDT detection was still high while mortality was very low. This detection increased with the amount of DDT applied. On this basis it was assumed that it was possible to increase the amount of DDT applied in the field to a level where a repellent effect could be observed. Such an experiment was performed and the amount necessary was beyond practical value. These results will be reported in a separate paper.

The decreases in detection indicated here may not be the result of a genetic change associated with exposure to DDT. These decreases are comparable to decreases in mortality and may be directly associated with the increase in surviving numbers. As the beetles become more tolerant to DDT, their behavior is less modified, so that they respond to any feeding damage on the treated leaflet in the same manner as they did in the case of physical damage to untreated leaflets. That DDT is still operative is indicated by the short length of time (280 minutes) in which physical damage reverses as compared with the little change that occurs in a longer period (72 hours) where the beetle chooses between treated leaf and untreated leaf.

It is assumed that detection of DDT is due to contact chemoreceptors, and that receptors are probably located on the tarsi and similar to those of the house flies (Dethier 1955). Potato flea beetles readily landed and walked on DDT treated leaflets, but the examination by maxillary and labial palpi of DDT-treated surfaces was not often observed. Therefore, the inhibition of the feeding mechanics as described in this paper and shown graphically by the reduced feeding on treated leaflets, demonstrated that rejection occurred after tarsal contact. Furthermore, this response in the absence of light indicates that this is not a visual response.

The situation here seems the reverse of the observations of Smyth & Roys (1955), in which susceptible house flies spent more time on DDT-treated surfaces than on untreated surfaces.

Study of the feeding behavior indicated that this insect exhibits a strong positive reaction to temperature, injured leaflet tissue, light, and upper leaflet surface. Light was shown to elicit a stronger response than upper leaflet surface. With light as an additional stimulus, it was possible to force the flea beetles to feed on the DDT-treated surface, however, DDT was also shown to interfere with response to light.

Table 4.—Effect of light on selection of feeding surface[a] by DDT resistant potato flea beetles.

Treatment	Number of Feeding Punctures		Per Cent Punctures in Upper Surface
	Lower Surface	Upper Surface	
Dark	156	301	65.9
150 f.c.	174	389	69.1
300 f.c.	54	275	83.6

[a] Normal leaf position.

Table 5.—Preferred feeding surface of potato flea beetles on leaflets of two potato varieties and effect of light on this preference.

Variety	Per Cent of Total Bites in Exposed Leaflet Surface	
	Upper Surface Exposed	Lower Surface Exposed
	In dark	
Katahdin	81.6	56.3
Irish Cobbler	76.9	49.8
	In light	
Katahdin	70.6	75.7
Irish Cobbler	81.7	66.8

REFERENCES CITED

Barnhart, C. S., and L. E. Chadwick. 1953. A "fly factor" in attractant studies. Science **117**: 104–5.

Dethier, V. G. 1955. The tarsal chemoreceptors of the house fly. Proc. Roy. Ent. Soc. Ser. A **30**(7–9): 87–90.

Dethier, V. G. 1956. Repellents. *In* Ann. Rev. of Ent. **1**: 181–202.

Fisher, E. H. 1957. Insect control in 1957. Wisconsin Agric. Expt. Sta. Circ. **520**: 35 pp.

Gould, G. E., and L. L. McCrosky. 1954. Potato flea beetle control with new insecticides. Jour. Econ. Ent. **47**(1): 190.

Grayson, J. M., and D. G. Cockran. 1955. On the nature of

insect resistance to insecticides. Virginia Jour. Sci. N.S. 6(3): 134–45.
Hofmaster, R. N. 1956. Flea beetle control on Irish potatoes in Eastern Virginia. Jour. Econ. Ent. 49(4): 530–3.
Jones, L. R. 1894. Spraying potatoes. Vermont Agric. Expt. Sta. Bull. 40: 25–6.
Kerr, T. W. 1957. Are potato flea beetles resistant to DDT? Rhode Island Agric. 3(2): 5; 10.
Kring, J. B. 1955. Control of DDT-resistant potato flea beetles. Connecticut Agric. Expt. Sta. Circ. 193: 23.
Kring, J. B. 1956. Dieldrin and endrin for control of DDT-resistant potato flea beetles. Jour. Econ. Ent. 49(4): 557–8.
Sleesman, J. P. 1955. Potato pest control. Market Growers' Jour. 84(4): 6–7.
Smyth, Jr. T., and C. C. Roys. 1955. Chemoreception in insects and the action of DDT. Biol. Bull. 108(1): 66–76.
Turner, N. 1934. Control of the potato flea beetle. *In* Connecticut Agric. Expt. Sta. Bull. 360: 445–6.
Turner, N. 1940. Biology and control of the potato flea beetle. *In* Connecticut Agric. Expt. Sta. Bull. 434: 277–83.
Turner, N., and N. Woodruff. 1946. Factors affecting the toxicity of DDT residues. *In* Connecticut Agric. Expt. Sta. Bull. 512: 93–7.

DDT-Resistance of the Colorado Potato Beetle[1]

L. K. CUTKOMP, A. G. PETERSON, and PRESTON E. HUNTER,[2] *Department of Entomology and Economic Zoology, University of Minnesota, St. Paul*

ABSTRACT

Field observations in the Red River Valley of Minnesota and North Dakota indicated some resistance of the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) to DDT as early as 1952 and 1953. This indication of resistance has been supported by determining the toxicity levels of injected DDT to adult beetles collected from two widely separated areas. Beetles were taken from the Red River Valley where resistance is apparent and from Belleville, Ontario, Canada where no DDT or chlorinated hydrocarbons have been used. The Minnesota beetles tolerated large doses of DDT. The LD-50 value was greater than 3000 micrograms per gram of body weight. The Canadian beetles were more susceptible, and the LD-50 values were 322 and 435 micrograms per gram for males and females, respectively. Since Dimite used in combination with DDT did not produce any detectable difference from DDT used alone, the results did not provide any clue as to the mechanism of the resistance.

The development and occurrence of tolerance or resistance of many species of insects and mites to insecticides has been summarized and reviewed by many workers (Babers 1949, Babers & Pratt 1951, Metcalf 1955, Hoskins & Gordon 1956). The tolerance to DDT constituted a major portion of the reports with the resistance of the house fly being prominent. Some agricultural pests have been included in the reviews.

The Colorado potato beetle was found to be very susceptible to DDT in the earliest work (Lauger *et al.* 1944), and its control under field conditions was demonstrated in the midforties in the United States by Granovsky (1944), Gyrisko *et al.* (1945) and others. After some years of extensive use of DDT in potato growing regions the beetles in certain areas were observed to be difficult to control with DDT. Quinton (1955) indicated the first appearance of resistance on Long Island, N. Y., in a small area in 1952 with areas expanding in 1953 and 1954. Post (1954) reported an apparent failure of DDT to control the Colorado potato beetle in a limited area of the Red River Valley in North Dakota during 1952, and there appeared to be increasing evidence for resistance in 1953. Resistance of the beetles to DDT in Europe is indicated (Kuhn & Löw 1955), but a full discussion of the situation has not been found in the literature.

During a brief survey made in August, 1954, a number of potato growers in the Crookston and East Grand Forks areas of the Red River Valley in northwestern Minnesota reported definite resistance of the beetle to DDT. Some of these growers could not obtain the desired degree of control by using DDT at rates as high as 2 pounds of actual DDT per acre. A field experiment was conducted at Crookston in 1955 to provide further information on the resistance of the Colorado potato beetle to DDT and to compare the relative effectiveness of DDT, toxaphene, heptachlor, and dieldrin.

Laboratory studies were planned to provide evidence, with known dosages, of differences in DDT susceptibilities between unrelated populations of Colorado potato beetles. During 1956, preliminary tests with beetles collected near Crookston, Minnesota, indicated that these beetles were difficult to kill with DDT; however, we could find no population of "DDT-susceptible" beetles elsewhere in Minnesota for comparison. In the potato-growing area near Hollandale, Minnesota, for example, the periodical applications of DDT apparently resulted in extermination of the beetles some 7 or 8 years ago. In 1957, Mr. Conrad C. Loan of the Science Service of the Canadian Department of Agriculture provided samples of "DDT-susceptible" beetles which he collected from a potato field near Belleville, Ontario, Canada. Only arsenicals had been used on this field, and, as far as he could determine, no DDT had been used on the potatoes in the vicinity.

FIELD OBSERVATIONS.—During 1955, DDT, toxaphene, dieldrin, and heptachlor were compared for their effectiveness in controlling the Colorado potato beetle in a field of Bliss Triumph potatoes grown at the North-

[1] Paper No. 3944 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota. Accepted for publication June 9, 1958.
[2] The help of Mr. Conrad C. Loan in obtaining beetles from Belleville, Ontario, is gratefully acknowledged. The writers also wish to express their appreciation to Mr. D. M. Noetzel, Mr. R. E. Hunt, Mr. A. M. Kolkaila, and to the staff of the Northwest School and Experiment Station, Crookston, Minnesota, for their assistance and cooperation.

west Experiment Station, Crookston, Minnesota. A randomized block design was used with four replications. Plots were each five rows wide by 30 feet long with an untreated buffer row between adjoining plots. Dust formulations were applied with a rotary hand duster at a rate of approximately 30 pounds per acre on June 21, July 10, and August 3.

Colorado potato beetles were moderately abundant in the plots at the time of the first application of insecticide, and there was an average of about 2% foliage injury. Egg masses were quite numerous at this time, and the eggs were beginning to hatch. Three days after the first application of the insecticides adult beetles were seen in only one of the four DDT plots, one of the toxaphene plots, one of the heptachlor plots, and all four of the untreated plots.

On July 10 a rating system was used to evaluate the treatments with respect to populations of potato beetles (chiefly larvae) and degree of injury. The different classes in the rating system were as follows: 1.—No potato beetles in entire plot and no injury. 2.—One to five beetles in plot and slight injury. 3.—More than five beetles in plot and injury present but no plants stripped. 4.—Beetles moderately abundant and occasional plants stripped. 5.—Beetles abundant and more than 10 plants stripped of foliage. According to the ratings, the DDT treatment was not significantly better than the check (table 1). Dieldrin, heptachlor, and toxaphene all had significantly lower ratings than either the DDT treatment or the check.

Heavy rains on July 13 beat the larvae off the plants and killed many of them. The new generation of adults began to emerge in late July. Counts of adults on July 26 indicated a fairly large population in the check plots, moderate numbers in the DDT plots, and few beetles in the dieldrin, heptachlor, and toxaphene plots. By August 2, the beetles had apparently distributed themselves rather evenly over all of the plots. Adult counts on August 4, the day after the final application of insecticides, indicated satisfactory control in all of the treated plots.

Both the June 21 and August 3 applications of DDT gave better control of the adult beetles than might be expected. This may have resulted from the combination of relatively heavy applications of DDT (1½ to 2 pounds actual per acre) and small populations of adult beetles. Under the conditions of this experiment, DDT gave practically no control of the Colorado potato beetle larvae.

Table 1.—Control of Colorado potato beetle with insecticides. Crookston, Minnesota, 1955.

Insecticide[a]	Rate (Lb./A.)	Control Rating July 10	Adults per Row July 26	Adults/4 Rows Aug. 2	Adults/4 Rows Aug. 4
DDT 5%	30	4.5	20.3[b]	17.5	1.0[c]
Toxaphene 5%	30	2.5[c]	6.0[c]	18.8	1.0[c]
Heptachlor 2½%	30	2.3[c]	3.5[c]	12.5	0.7[c]
Heptachlor 2½%	50	1.8[c]	3.5[c]	15.0	0.3[c]
Dieldrin 1½%	30	1.5[c]	3.5[c]	19.3	1.3[c]
Check	—	5.0	46.3	11.0	9.5

[a] Applied June 21, July 10, and Aug. 3.
[b] Significantly lower than check (5% level).
[c] Significantly lower than check (1% level).

This is indicated in the control rating of July 10 (table 1) which was based upon the presence of larvae and on the extent of larval feeding. The results obtained in 1955 are in contrast with results in 1949 which indicated that dieldrin, aldrin, and several formulations of DDT were all equally effective in controlling the Colorado potato beetle (Peterson 1950).

LABORATORY EXPERIMENTS.—During July, 1957, adult beetles were collected from two potato fields near Hendrum, Minnesota, and one field near Crookston, Minnesota. Both areas are in the Red River Valley where beetles have apparently developed resistance to DDT. At approximately the same time, adult beetles were received from Canada. The different lots of beetles were kept on potatoes in field cages prior to their use in the laboratory.

Methods and Materials.—Each test beetle was injected with olive oil as a solvent or with known quantities of DDT in olive oil. Two or three cubic millimeters of liquid were injected between the abdominal sternites and slightly to the side of the mid-ventral line to avoid puncture of the gut. A No. 27 hypodermic needle was used for injection. Following injection the beetles were placed individually in salve tins with a potato leaf for food and paper towelling to absorb excess moisture. The treated beetles were placed in constant temperature cabinets. The majority of the beetles were kept at a post-treatment temperature of 25° C., but some were kept at 15° C. to determine if a different effect might result. Mortality determinations were made each day for at least 7 days, and in some cases observations were continued for 2 weeks or more. Fresh leaves were given to the survivors.

Determinations of the sex of the beetles was made on the basis of a difference in shape of the fifth visible abdominal sternite. The hind margin of this sternite on males was slightly biconcave with a small protruding process in the middle. In females the hind margin was broadly convex (rounded). Dissections were made of over 50 beetles to verify the anatomical difference.[3] Further information on this difference and on comparative weights will be published as a separate note.

Results.—High concentrations of DDT injected into Minnesota beetles produced less than 35% mortality in the females and less than 45% in the males. Beetles from Crookston, Minnesota, received doses ranging from 610 to 2310 micrograms per gram for females and 610 to 2500 micrograms per gram for males. Of the 60 females treated, 16 died from widely scattered doses, and of the 36 males treated, 11 died from widely scattered doses. No reliable minimal or median lethal dose could be determined (table 2). The per cent mortality of beetles from Hendrum, Minnesota, was also too low for any reliable estimate, with three of 10 beetles dying from doses of 2090 to 3000 micrograms per gram of body weight. This was the maximum dose that could be administered with the solvent and the method used.

The effect of lower post-treatment temperatures was investigated. A high dose of 450 micrograms DDT per beetle resulted in 60 and 50% mortality at post-treatment temperatures of 15° and 20° C., respectively. Ten beetles were used for each temperature treatment, but the

[3] Mr. C. S. Li of the Department of Entomology and Economic Zoology of the University of Minnesota was responsible for information on this anatomical feature. An early description was given on p. 33 of Tower (1918).

Table 2.—Mortalities of Minnesota beetles injected with DDT dissolved in olive oil.[a]

Sex	DDT Dosage	No. Injected	No. Dead	Per Cent Mortality
Females	0	9	0	0
	798.7	15	5	33
	1,171.3	15	4	27
	1,499.3	15	3	20
	2,049.3	15	4	27
Males	0	10	1	10
	843.3	9	4	44
	1,454.4	9	2	22
	2,038.9	9	3	33
	2,391.1	9	2	22

[a] DDT dosage is expressed as mean micrograms of DDT per gram of body weight for each group of beetles. Post-treatment temperature 25° C.

differences were not significant and an accurate determination of medial lethal dose was not possible. In summarizing the results pertaining to beetles from Minnesota, the indications are that the LD-50 is greater than 3000 micrograms per gram (3 micrograms per milligram) of body weight to both sexes.

Because the Minnesota beetles showed a high degree of tolerance to DDT, one additional experiment was conducted in which a combination of Dimite with DDT was injected. This was done to determine if the DDT dehydrochlorination could be inhibited or minimized as it is in resistant strains of house flies (Perry *et al.* 1953, Moorefield & Kearns 1955, Cohen & Tahori 1957). There was no indication that Dimite was useful in this respect with Colorado potato beetles. Evidence for this came from results of injecting Minnesota beetles with equal quantities of Dimite and DDT. Equal doses of 100 to 188 micrograms of each compound were injected per beetle (540 to 1430 micrograms of DDT per gram of beetle weight), and only five of the 36 beetles died. A similar level of mortality occurred among controls injected with olive oil alone. If the Dimite had been an effective inhibitor of DDT degradation, the per cent mortality would have been higher.

Beetles presumed to be susceptible to DDT were studied for their responses to DDT. These were Canadian beetles collected from an area where no chlorinated hydrocarbons had been used. The dosage mortality determinations are presented in figure 1. They are based upon responses of 80 beetles. LD-50 values of 322 micrograms per gram and 435 micrograms per gram were calculated for males and females, respectively, when kept at 25° C. post-treatment temperature. The dosage mortality curves for males and females were parallel, and the difference in LD-50 values was significant. Few beetles were tested at the 15° C. post-treatment temperature, and considerable variation in the results at this temperature made calculation of the LD-50 values difficult. However, inspection of the data did not indicate an appreciable change in response due to temperature.

Discussion.—Field observations made in the Red River Valley of Minnesota and North Dakota indicating the occurrence of tolerance or resistance of Colorado potato beetles to DDT are now supported with studies of the response of beetles to injected DDT. The resistance is great, but a precise level was not determined. It is suggested that the beetles are able to tolerate doses at least 10 times greater than those tolerated by the beetles sampled from Ontario, Canada.

Because the resistance was relatively high the additional experiment with Dimite with DDT was carried out to provide a lead as to whether the dehydrochlorination of DDT could be important and could be inhibited. In resistant house flies the presence of Dimite acts as a competitor of DDT with respect to the process of dehydrochlorination. The resultant effect is that a lower dose of DDT will produce mortality. In this study high concentrations used did not produce any greater mortality among the test beetles than among the controls. From these negative results one can only conclude that no information was obtained on the mechanism of resistance among DDT-resistant beetles or whether detoxification processes differ qualitatively or quantitatively in the two unrelated populations of beetles studied.

Results obtained from the presumably susceptible beetles from Canada were somewhat unexpected in that rather high doses of DDT were required to produce mortality. Although still far below the doses necessary to kill

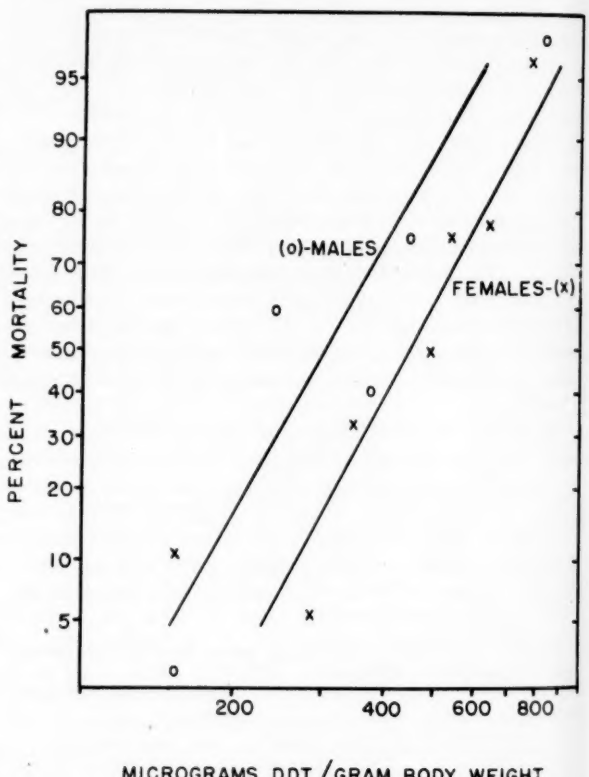


Fig. 1.—Dosage-mortality curves for Colorado potato beetle adults collected near Belleville, Ontario, Canada, with presumably no history of DDT use. Beetles were injected with DDT in olive oil. LD-50 values for males were 322 (228 to 454) micrograms of DDT per gram of body weight and for females 435 (346 to 557) micrograms of DDT per gram. The values in parenthesis represent the confidence limits of the LD-50. The LD-50 values differed significantly between sexes at the 5% level of significance.

the Minnesota beetles, the doses are considerably higher than for most DDT-sensitive beetles. Perhaps the larval stages are much more sensitive, or perhaps a high stomach-contact toxicity accounts for the fact that susceptible strains are easily killed by field applications of DDT.

The present laboratory study has not included comparisons with other insecticides. Other insecticides used to replace DDT for control of resistant beetles are presented by Quinton (1955) and given in Minnesota state recommendations (Anonymous 1958).

The resistance to DDT apparently developed in relatively few generations of the beetle as compared with numbers of generations reported for some other species of insects, *e.g.*, the house fly. Some degree of resistance developed during the short period from 1946 to 1953, involving a minimum of seven to a maximum of 14 generations of the beetle.

REFERENCES CITED

Anonymous. 1958. Commercial vegetable insect and disease control guide. Agric. Ext. Serv., Inst. of Agric., Univ. of Minnesota. Multilithed.

Babers, F. H. 1949. Development of insect resistance to insecticides. U. S. Dept. Agric., Agric. Res. Admin. **E-776,** 31 pp.

Babers, F. H., and J. J. Pratt, Jr. 1951. Development of insect resistance to insecticides II. U. S. Dept. Agric., Agric. Res. Admin. **E-818,** 44 pp.

Cohen, S., and A. S. Tahori. 1957. Mode of action of di-(p-chlorophenyl)-(trifluoromethyl)-carbinol, as a synergist to DDT against DDT-resistant houseflies. Jour. Agric. and Food Chem. **5:** 519–23.

Granovsky, A. A. 1944. Tests of DDT for the control of potato insects. Jour. Econ. Ent. **37:** 493–9.

Gyrisko, G. G., J. F. T. Jodka, and W. A. Rawlins. 1945. DDT to control potato insects. Jour. Econ. Ent. **38:** 169–73.

Hoskins, W. M., and H. T. Gordon. 1956. Arthropod resistance to chemicals. Ann. Rev. of Ent. **1:** 89–122.

Kuhn, Richard, and Irmentrant Löw. 1955. Resistance factors against *Leptinotarsa decemlineata* Say, isolated from the leaves of wild *Solanum* species. *In* Origins of Resistance to Toxic Agents. pp. 122–32. Academic Press Inc., New York, N. Y.

Läuger, P., H. Martin, and P. Müller. 1944. Über Konstitution und toxische Wirkung von natürlichen and neuen synthetischen insektentötenden Stoffen. Helvetica Chim. Acta **27:** 892–928.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. Jour. Pharmacol. and Exptl. Therap. **96:** 99–113.

Moorefield, H., and C. W. Kearns. 1955. Mechanism of action of certain synergists for DDT against resistant house flies. Jour. Econ. Ent. **48:** 403–6.

Perry, A. S., Mattson, A. M., and Buckner, A. J. 1953. The mechanism of synergistic action of DMC with DDT against resistant house flies. Biol. Bull. **104:** 426–38.

Peterson, A. G. 1950. Insecticide experiments on potatoes in Minnesota during 1949. Amer. Potato Jour. **27:** 319–26.

Post, R. L. 1954. "DDT-resistant" Colorado potato beetles? Proc. North Central Branch Ent. Soc. America **9:** 94–5.

Quinton, R. J. 1955. A study of DDT resistance in the Colorado potato beetle *Leptinotarsa decemlineata* Say. Ph.D. Thesis, Cornell Univ., Ithaca, New York.

Tower, W. L. 1918. The mechanism of evolution in *Leptinotarsa.* Carnegie Institution of Washington. Publ. No. **263.**

Public Health Hazards Involved in the Use of Organic Phosphorus Insecticides in Cotton Culture in the Delta Area of Mississippi[1]

GRIFFITH E. QUINBY, KENNETH C. WALKER, and WILLIAM F. DURHAM

ABSTRACT

During the summer of 1957, suspected cases of poisoning by cotton insecticides were investigated in the Mississippi Delta area. Blood cholinesterase levels of cases and of workers with maximal exposure to organic phosphorus insecticides were determined. In addition, the decay rates of insecticides and the exposure of workers were measured.

Ninety-one illnesses suspected of being poisoning due to insecticides were evaluated. Evidence was found to definitely incriminate organic phosphorus compounds as a cause of illness in two workers in formulating plants. A diagnosis of poisoning must be considered in three pilots. One fatality and one other illness were attributed to the ingestion of chlorinated hydrocarbons. In 7 of 11 crashes or disturbances of the pilot's performance about which enough information was available, three appeared to represent pilot error, one was myocardial infarction, and three—mentioned above—appeared to be mild poisoning with consequent interference with flying ability.

An epidemic characterized by fever and respiratory symptoms accounted for the majority of cases suspected of being poisoning. Epidemiologic and toxicologic investigation of this epidemic established infection and ruled out insecticides as the cause of these illnesses.

Groups of persons selected because of their exposures to methyl parathion or to Guthion resulting from their occupation or from their residing in treated areas were studied for evidence of poisoning. No clinical evidence of poisoning or significant cholinesterase depletion was found.

The half-life for methyl parathion on cotton leaves was found to be less than 1 hour. Exposure studies on checkers corroborated this rapid decay rate. Most of the residue exposure was on the hands.

The half-life of Guthion spray and dust on cotton leaves appeared to be about 2 to 4 days. Exposure studies indicated that most of the residue exposure was to the hands.

The reported failure of chlorinated hydrocarbon insecticides to achieve satisfactory control of the boll weevil in the Mississippi Delta in 1955 led to the increased use of

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Wenatchee, Washington. This paper was endorsed and communicated for publication by K. D. Quarterman. Accepted for publication June 13, 1958.

Table 1.—Application rate, concentration, and length of cycle of organic phosphorus insecticides as used in the Delta area of Mississippi in 1957.

Insecticide	Application Rate (Lb./Acre)	Concentration (Per Cent)					Length of Cycle (Days)
		Spray			Dust		
		Emulsifiable Concentrate	Dilute				
			Ground	Airplane	Ground	Airplane	
Malathion	0.5 –1.0	65	1 –2	6–12	4 –10	4 –10	3–5
Methyl parathion	0.25–0.50	25	0.5–1.5	3–6	2.5– 5.0	2.5– 5.0	3–5
Guthion	0.25	20	0.5	3	—	—	5–7

organic phosphorus compounds, especially malathion [*O,O*-dimethyl S-(1,2-dicarbethoxyethyl)dithiophosphate] and methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate) (Furr & Lloyd 1957). An even greater use of methyl parathion and Guthion [S-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate] was anticipated for 1957. It was believed by some in Mississippi that poisoning had occurred and was occurring in both occupationally exposed persons and in residents of the area where these insecticides were being used. The idea also prevailed that the poisoning of pilots was responsible for an alleged increase in the airplane accident rate.

Various state and federal agricultural agencies in the Mississippi Delta area requested the Communicable Disease Center of the Public Health Service to investigate the hazards associated with the use of these insecticides. This report presents the results of the study.

The introduction of new or more toxic insecticides into an area has often led to fears for the safety of the populace. Sumerford *et al.* (1953) in 1951 studied cholinesterase response and symptomatology from exposure to organic phosphorus insecticides in the fruit orchards of North Central Washington. The survey was continued in 1952 and 1953 (Hayes *et al.* 1957). There was, at the beginning, considerable anxiety in the community because of deaths and illnesses attributed to minimal exposure to these compounds. It was found that persons definitely exposed to parathion showed a significant reduction in average cholinesterase values and an increase in illnesses consistent with poisoning during the period of their exposure. No evidence was found of either reduced cholinesterase activity or increased illness during the spray season in persons with little exposure to insecticides, including those who merely lived within the treated area.

Under somewhat similar conditions, Fowler (1953) investigated in 1952 the health hazards involved in the use of the newer economic poisons in the control of cotton insects in the Mississippi Delta. At that time the chlorinated hydrocarbon compounds were of most concern. Although he reported seven cases of acute insecticide poisoning following extensive exposure, Fowler found no evidence of any chronic intoxication by insecticides and no indication that they acted as a contributing cause in diseases generally recognized as having other etiologies.

Quinby & Lemmon (1958) have recently described outbreaks of poisonings in crop workers from dermal exposure to parathion residues on foliage and fruit. Agricultural authorities in Mississippi were familiar with these findings and were, therefore, fully aware that poisoning by at least one organic phosphorus insecticide was possible through exposure to residues as well as through contamination during application.

Conditions of Study.—The problem in the Delta area was approached in four ways: (1) Suspected cases of poisoning were investigated. (2) Blood cholinesterase levels of cases and of workers with maximal exposure to organic phosphorus insectidices were determined. (3) The decay rates of insecticide residues on cotton were followed. (4) The respiratory and dermal exposure of cotton checkers was measured.

The studies were carried out during the summer of

Table 2.—Evaluation of reported cases suspected of being poisoning by cotton insecticides, Mississippi Delta area, 1954–57.

Evaluation[a]	Organic Phosphorus Insecticides					Chlorinated Hydrocarbons	Unspecified Insecticides	Total
	Parathion	Methyl Parathion	Guthion	Malathion	Unspecified			
Fatal Poisoning	0	0	0	0	0	1 (Endrin)	0	1
Non-fatal Poisoning:								
Cholinesterase confirmed	0	0	0	0	0	—	—	0
Clinically consistent only	2	1	1	1	0	1	0	6
Asymptomatic cholinesterase depletion	0	3	0	0	0	—	—	3
Doubtful poisoning	1	2	0	0	0	0	0	3
Exposed, illness unrelated	0	21[b]	1	13	2	0	4	41
Unexposed, illness unrelated	1	28[b]	0	0	6	0	2	37
Insufficient data	0	2	0	0	2	2	3	9
Total	4	57	2	14	10	4	9	100

[a] Tabulated in order of relative certainty of poisoning.
[b] Epidemic respiratory diseases.

1957 from headquarters at the Delta Branch Experiment Station, Stoneville, Mississippi. In addition to intensive investigations carried out at the Station and at selected places nearby, reports were solicited from 13 counties in West Central Mississippi which included an area limited approximately by Greenville, Clarksdale, Greenwood, and Vicksburg.

All practicing physicians in these 13 Delta counties were advised of the study by letter and requested to report all cases of suspected poisoning; laboratory tests and other assistance were offered in connection with these cases. A technical review of the toxicology of the organic phosphorus insecticides with emphasis on early recognition, laboratory confirmation, and treatment was presented before the staff of each of 10 hospitals in the area. During the series of visits to each hospital, their records since 1954 were searched for past and current cases of poisoning. The health departments of the nearby states were requested to report any cases of insecticide poisoning and were alerted to watch especially for residue contact poisoning in agriculture workers. A few individual cases were reported from these states and are included in the tabulations.

As a result of these activities, a total of 100 cases of suspected insecticide poisoning were studied, including 11 episodes involving either an airplane crash or a disturbance of a pilot's performance.

During the course of the clinical investigations and planned blood cholinesterase surveys, the cholinesterase activity values of about 800 blood samples were generously made available by private laboratories. There were multiple samples on some persons. In addition, 131 blood samples including all those shown later in table 3 were analyzed at the Technical Development Laboratories, Savannah, Georgia. All cholinesterase determinations were carried out by the Michel procedure (Michel 1949) unless specified otherwise.

Ninety-two persons representing eight occupational groups with varying levels of exposure to methyl parathion were studied to determine the effect of this exposure on their health. Exposure and clinical histories were obtained from all 92 persons and cholinesterase activity levels were determined. An effort was made to include within each group those persons with the heaviest exposure. In a similar manner, 32 persons were studied in a survey on Guthion. Since malathion has a very low mammalian toxicity, no special exposure studies were made on this compound but, of course, alleged poisonings were investigated.

In preparation for studies of the decay rates of insecticide residues and also for measurements of the exposure of cotton checkers, insecticide applications were made by the staff of the Delta Branch Experiment Station, Stoneville, Miss., using ground equipment. A machine similar to commercial ones but especially designed for precise application was used. For each compound, residue decay determinations and exposure studies were carried out concurrently in the same plot. During this work, air temperatures taken near the leaves ranged from 59 to 97° F. and the relative humidity was high.

After the spray had dried (less than 5 minutes) or the dust had settled, leaves were removed and placed in a new paper bag. From cotton in the various rows, leaves were removed from representative positions on the top and middle canopy of the plants and some from the interior, but none from the lower third near the ground.

The time interval between the removal of the leaves from the plant and their extraction was held to a minimum. The decay time represents the interval between application and extraction. All references to leaf residue are expressed in terms of square inches of leaf, not leaf surfaces. Most of the samples were prepared by cutting 1 sq. in. from each of 50 leaves stacked together. The cuttings were immediately placed in a reagent bottle and washed with three serial rinsings of approximately 100 ml. of ethyl alcohol. The bottle was shaken vigorously 20 times in about 30 seconds for each rinsing. The alcohol extract was mailed to the laboratory 2 to 3 days away. Recovery from control samples of methyl parathion shipped in the

Table 3.—Mean blood cholinesterase activities of occupational groups arranged in estimated order of decreasing exposure to methyl parathion or to Guthion.

Occupational Group	Health Status[a]	No. of Persons	Average Cholinesterase Activity (ΔpH/Hr.)	
			R.B.C.	Plasma
Exposure to methyl parathion				
Loaders	Well	6	0.62	0.84
Applicators	Sick	4	.77	.85
	Well	1	.89	1.20
Warehousemen	Sick	1	.65	1.16
Pilots	Sick	2	.83	.83
	Well	4	.62	1.07
Experimental entomologists	Well	4	.71	.69
Checkers	Well	21	.78	.92
Choppers or hoe-hands	Sick	3	.63	.38
	Well	10	.72	.90
Residents	Sick	26	.72	.86
	Well	10	.72	.97
Total or mean	Sick	36	.72	.83
	Well	56	.73	.92
Exposure to Guthion				
Experimental entomologist	Well	1	0.75	0.58
Flagmen	Well	3	.76	.88
Pilot	Sick	1	.88	.82
Irrigators	Well	11	.77	.77
Checkers	Well	4	.73	.94
Field supervisors	Well	4	.83	1.08
Residents	Well	8	.79	.96
Total or mean	Sick	1	.88	.82
	Well	31	.78	.88

[a] Respiratory diseases were epidemic.

same way indicated no significant decay en route.

Methyl parathion was determined by the method of Averell & Norris (1948) which produces a magenta color. Some 2,-3,-and 4-day-old residues on leaves gave varying shades of brownish-magenta on final color development. The brown component probably affected the final optical density and tended to give values slightly higher than true values. These values are interpreted as of questionable accuracy. In general, these values were not more than 50 micrograms per total sample. Samples collected 5 or more days after spraying gave no detectable magenta on final color development and the values calculated from the optical densities were below the limit of sensitivity of the method (20 micrograms). A blank value of 13 micrograms was used to correct all analyses of leaves.

Exposure studies were carried out using techniques similar to those described by Batchelor & Walker (1954) and by Batchelor *et al.* (1956). Recovery studies were carried out to estimate the possible loss of sample from exposed garments during the mailing time of 2 to 3 days. Similar garments were treated at the laboratory with known amounts of methyl parathion, saturated with saline to simulate perspiration, and stored under even hotter conditions than those to which air mail would subject them. Losses were insignificant from the dry specimens but as high as 50% from wet ones.

Guthion was determined by a modification of an unpublished method furnished by the Chemagro Corporation.

Spray practices concerned with the control of cotton insects in the Mississippi Delta during 1957 may be summarized as follows:

The proportion of cotton growers in the Delta area using organic phosphorus insecticides varied from less than a third at the beginning of the 1957 season to over half at the end. Early, malathion was by far the most commonly used organic phosphorous compound, but by the end of the season the use of methyl parathion had increased until it probably exceeded malathion, at least in acreage covered. A smaller amount of Guthion was used. Parathion (*O,O*-diethyl-*O*-*p*-nitrophenyl thiophosphate), demeton [*O,O*-diethyl S-(2-ethylthio) ethyl phosphorothioate mixture with *O,O*-diethyl *O*-(2-ethylthio) ethyl phosphorothioate], and other organic phosphorus insecticides were rarely employed.

The ecology of the boll weevil is such that control has been obtained by spraying or dusting the canopy of the cotton fields. Sprays have been more popular than dusts. The application rate, concentration, and length of cycle for organic phosphorus insecticides used in the Delta area of Mississippi are shown in table 1. The volume rate of mixed spray was about 5 to 6 gallons per acre by ground equipment and about 1 gallon per acre by airplane. Control of various combinations of cotton pests frequently required that mixtures of insecticides be applied at these same low volumes per acre. Some applications of larger than recommended amounts of insecticide per acre were made. Both of these practices sometimes resulted in an increased concentration of petroleum distillate vehicle in the finished spray. Dust applications ranged from 5 to 20 pounds of diluted material per acre.

Early in the 1957 spray season, two statements on safety precautions had been printed by the Delta Council and the Mississippi Aeronautics Commission. A series of newspaper articles on safety had been sponsored by the Agricultural Extension Service. A supplementary statement on first-aid was issued later in the newspapers by the State Board of Health and the State Medical Association. In spite of these warnings, violations of recommended safety precautions were common. Often there was a practical reason for these violations, but some were readily preventable.

The more important of these safety violations were as follows:

1. Washing, bathing, and laundering facilities were generally lacking or inadequate.
2. Many pilots loaded their own planes.
3. Pilots usually did not cut off their motors during reloading.
4. Opened and "empty" spray containers were left accessible to children and other uninformed persons.
5. Discarded 5-gallon concentrate containers and dust sacks were used as permanent swath-markers or flags.
6. Loading and storage facilities for insecticides were unsafe.
7. Hoses connected to drinking water supply were placed into the spray mix.
8. The real toxic hazards of insecticides were concealed or minimized by some growers and sales agents.
9. There was a failure to provide for the blood cholinesterase testing of some pilots, loaders, and other handlers when they were recognized to have had spillage accidents or other excessive exposure.

It was observed that, in the course of these violations and in accidents, many workers tolerated heavy exposures to the more commonly used insecticides, malathion and methyl parathion, without developing symptoms.

RESULTS.—*Clinical Investigations.*—Of the 100 suspected cases of poisoning on which some data were available, enough was learned about 91 cases to evaluate them. These cases are tabulated in table 2 in order of their relative certainty of poisoning and on the basis of compound originally suspected.

No fatal case of organic phosphorus poisoning was found during the period covered by the study (1954–57). A child did die, apparently from ingesting endrin. The evidence indicates the child swallowed enough endrin from a can to cause convulsions and death.

Two nonfatal cases clinically consistent with parathion poisoning occurred in 1954 and 1955 in formulating plants. Cholinesterase tests were not made in these cases. In this connection, it should be noted that the volume of parathion formulated and used was small in former years and almost nil in 1957. By contrast, the amount of methyl parathion formulated and applied in the Delta area during 1956 and 1957 was considerable.

Three cases consistent with mild organic phosphorus insecticide poisoning sufficient to interfere with the efficiency of pilots are described below in detail; they were due to methyl parathion, Guthion, and malathion, respectively. One case consistent with poisoning from ingesting a mixture of BHC and DDT was also reported.

Asymptomatic cholinesterase depletion was found in three loaders. Two of them who had violated safety instructions had red blood cell cholinesterase activity levels below the usual symptomatic level for persons with small but repeated exposure. The third loader's post-exposure level was in the low normal range but was significantly below his pre-exposure values.

Three cases (1 parathion and 2 methyl parathion) were classified as doubtful. Their clinical pictures and exposure histories were not consistent with poisoning, but tests had not been done to rule out poisoning with certainty.

There were 78 persons each of whose illness was suspected of being poisoning but was considered after study to have some other cause. The histories indicated that 41 of these persons were workers in cotton fields or had been exposed in some other way. The remaining 37 were essentially unexposed. Many of these cases were ill in epidemics during the summer of 1957. Only one physician attributed any substantial number of these illnesses to poisoning. However, many people were concerned over the claims of this one physician that the epidemics were due to poisoning, especially when widespread newspaper and radio publicity was given to this possibility. Poisoning was reasonably ruled out by case histories and blood cholinesterase tests in one epidemic. In addition to the toxicologic studies in this particular area, the Mississippi State Board of Health sent an epidemiologic team to collect specimens and symptom data. Their findings gave direct support to the conclusion that this epidemic was caused by respiratory infections. Less intensive toxicologic surveys were conducted in two other smaller population groups suffering similar waves of respiratory disease. Neither of these surveys showed any indication of poisoning.

Several other reports not connected with the epidemic involved mistakes in initial diagnosis. It is not surprising that the public became alarmed in view of the extent to which stories based on rumors and on misdiagnosis were bandied around in the newspapers, by radio, and by word of mouth. These misconceptions reached their acme when the confusion between poisoning and the epidemic respiratory disease caused the etiologic agent of the epidemic to be called the "cotton poisoning virus."

Cases involving pilots are of special interest because pilots may be exposed to considerable contamination and because minor poisoning of a pilot might have far more serious consequences then similar poisoning of a person whose life did not depend on the skillful manipulation of an intricate machine. Eleven instances of airplane crashes or disturbances of a pilot's behavior were investigated. One occurred in 1955, three in 1956, and seven in 1957. Unfortunately, so little information was obtained in four of the cases that no decision whatever can be made regarding their probable causes. In two cases, it is not even established that the pilots were exposed to organic phosphorus insecticides. Three cases appeared to be clear examples of pilot error with the possibility of poisoning ruled out by adequate information. One case was myocardial infarction.

A diagnosis of poisoning must be considered in three of the cases which involved significant exposure and incoordination or other symptoms. The fact that blood cholinesterase values were normal shortly after the accidents offers no support to this view, but it in no way rules out local effects (Upholt *et al.* 1956) or even mild systemic poisoning (Sumerford *et al.* 1953). Obviously, mild intoxication or the visual disturbances caused by the local (or systemic) action of certain organic phosphorus insecticides would be sufficient to interfere with a pilot's efficiency. The three cases were:

R.W.: This pilot crashed in 1956 while carrying malathion dust, but he had reportedly been "affected" by methyl parathion some weeks previously. While his many fractures and other severe injuries adequately explained his death, they did not explain the erratic flying which a witness observed preceding the crash. A post-mortem Michel blood cholinesterase test was reported as normal (RBC, 81%; plasma 79%); however, the effect of earlier transfusions on this result cannot be stated.

F.R.: This 42-year-old pilot did not spray his load of methyl parathion and had difficulty landing on August 12, 1957 because he was dizzy, unable to think clearly, and had double vision. He vomited while still in the air. There was no exposure to any other organic phosphorus insecticide. The pilot frequently had seen spillage into his cockpit and specifically recalled gross exposure 1 and 5 days earlier. He had worked long hours and eaten at irregular intervals. A screening blood cholinesterase test by a hospital laboratory showed about 66% of normal on July 11 and about 42% of normal on August 12, the day of his landing difficulty. However, by the Michel procedure, his level was normal on this date (RBC, 0.79; plasma, 0.83 ΔpH/hr.). The skin flushed following a single dose of atropine which argues against severe systemic poisoning. At the time of physical examination, no pupillary changes were noted. However, the most interesting feature of the case was that the pilot found that his visual disturbance was corrected when he shut one eye. In this way, he was able to see clearly and land his plane safely. This reaction strongly suggests the imbalance of vision which can be caused by the local action of organic phosphorus insecticides (Upholt *et al.* 1956).

J.M.: This pilot, on August 20, 1957, had spilled Guthion concentrate on his hands but washed it off within 3 minutes. During the previous 10 days, he had been loading methyl parathion and had been contaminated to the point that the white dust could be seen on his skin. Following the spillage on August 20, he had visual disturbances, headache, tightness in the chest, abdominal cramps, nausea, vomiting and weakness, and some excess salivation while flying. He had difficulty in landing but did not crash. After landing, he was improved by a dose of atropine but still showed signs of hyperperistalsis on physical examination. All of these circumstances pointed to poisoning. He had had a sore throat for 5 days, but respiratory disease was epidemic in the area at the time. However, there was no evidence that the symptoms listed above were related to respiratory disease. The fact that the pilot never developed muscle fasciculations or pin-point pupils and was found to have a normal blood cholinesterase level (RBC, 0.88; plasma, 0.82 ΔpH/hr.) argued against severe systemic poisoning.

Blood Cholinesterase Survey.—The respiratory epidemics afforded an excellent opportunity to compare occupationally exposed groups and residents, both sick and well, in areas of heavy and frequent application of methyl parathion. The staff of the Delta Branch Experiment Station furnished representatives of the several groups (including loaders, applicators, pilots, research entomologists, and many checkers). Additional subjects in these occupational groups as well as choppers and one warehouseman were obtained from cotton plantations not associated with the agricultural tests of the station. An additional group of the most heavily exposed commercial checkers were tested. These checkers walked through cotton sometimes from dawn to dark without certain knowledge of the age or nature of the residues. Their clothing became soaked from the dew-wet, residue-laden leaves. At times they crawled on their hands and knees to search for weevils. Although nearly all these

commercial checkers had occasional exposures to Guthion residues and frequent exposures to malathion, they were classified as primarily exposed to methyl parathion on the basis that it was their most hazardous skin exposure. They had been concerned enough about their risk to have had pre-exposure blood tests done at their own expense.

The results of the blood tests of these occupational groups are shown in table 3. All of the 92 persons had values within the range of normal except for two loaders and one chopper. The two loaders had violated safety precautions; they were previously referred to under the section on clinical investigations. The one chopper with a definitely depleted plasma cholinesterase level was a hypertensive pregnant woman ill with respiratory disease. Her red cell cholinesterase activity was normal. She may have suffered liver damage from several toxemias of pregnancies.

It may be seen from table 3 that there was no appreciable difference in average cholinesterase level between the sick and the well persons within each occupational group, or between the sick and the well persons for the entire population sampled.

Only one plantation was found where the application of Guthion was sufficient to warrant conducting a blood survey. The blood testing was done on the sixth day of the fifth cycle on a 7-day aerial-spray schedule. A Guthion residue of 1.4 micrograms/sq. in. of cotton leaf was found at this time. Because of their substantial exposure to each compound, three individuals (a pilot, a checker, and a research entomologist) from the methyl parathion study were also included in the Guthion study. The residents lived in houses which were surrounded on three sides by cotton. Several choppers or irrigators also lived in houses in the treated cotton fields.

As can be seen in table 3, the mean cholinesterase values for each occupational group were within the range of normal, as were all individuals who made up the group.

Hundreds of other individuals from the same occupational groups were being tested by three private laboratories during and after the surveys summarized in table 3. As results from the private laboratories were made available, the 12 persons having levels below 0.50 ΔpH/hr. were studied in more detail with regard to clinical and exposure history. These low values occurred in less than 2% of the persons tested and the clinical and exposure histories ruled out symptomatic poisoning from organic phosphorus insecticides in all 12.

Residue and Exposure Studies.—The decay curves for two trials with methyl parathion residues are shown in figure 1. There is good agreement between values in the two trials. It appears from these curves that, under the conditions studied, the decay of methyl parathion on cotton leaves was quite rapid. The half-life appears to be less than 1 hour.

This rapid decay rate appears to be in reasonable conformity with previously reported rate of loss of residues of methyl parathion. Thus, Fahey *et al.* (1952) compared the longevity of parathion, methyl parathion, and EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate) on apple foliage in 1952. They found that the 24-hour residues of methyl parathion were 30% of the 1-hour baseline residue.

One possible explanation of the rapid decay rate noted

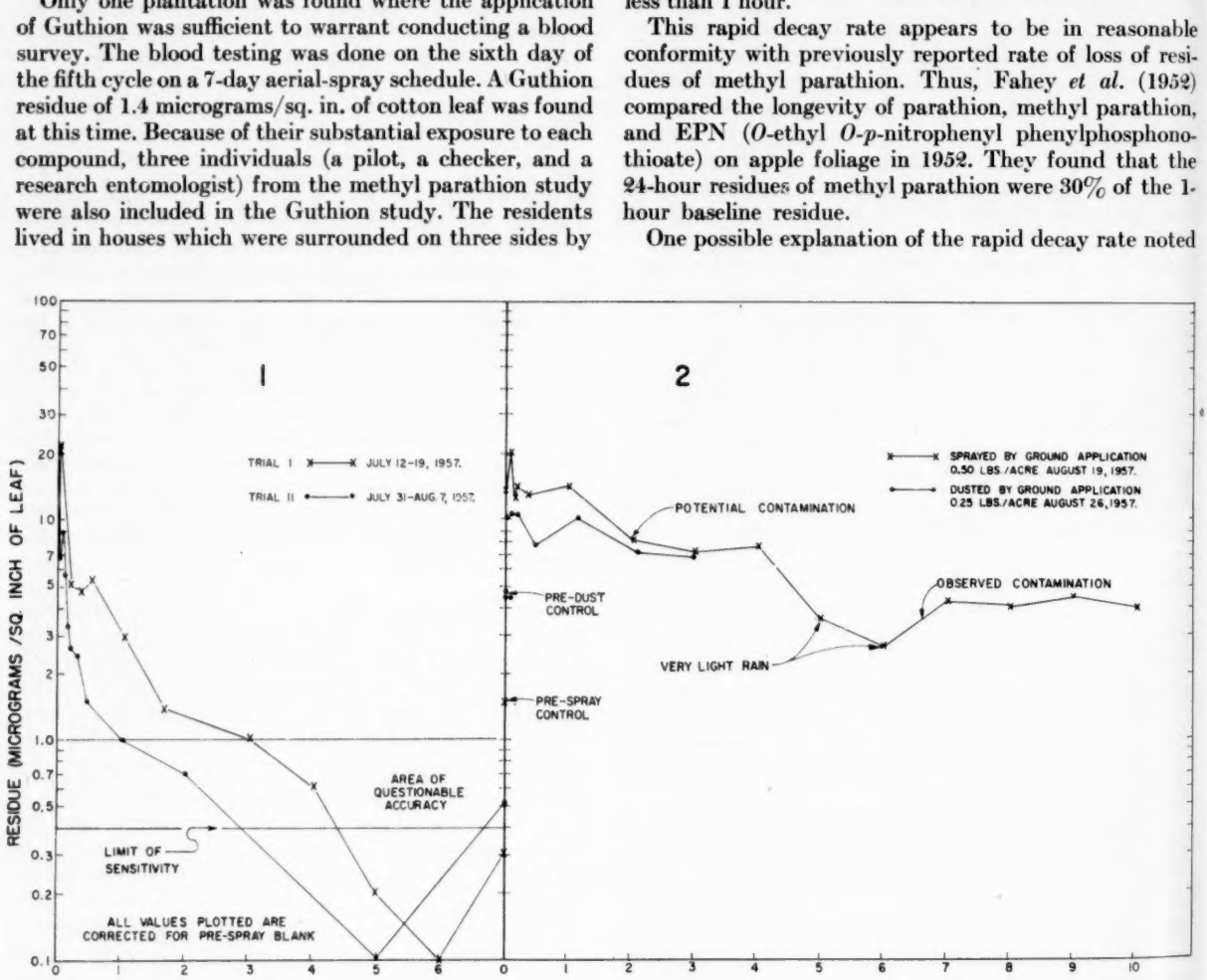


FIG. 1.—Decay rates of methyl parathion sprayed by ground application on cotton at 0.5 lb./acre (59 to 97° F.).
FIG. 2.—Decay rates of Guthion (59 to 97° F.).

Table 4.—Exposure of various body parts of checkers to recent ground or airplane application of methyl parathion spray to cotton.

	Ground (0.5 Lb./Acre)						Airplane (0.37 Lb./Acre)	
	Age of Residue						Aged 24–25 Hours	
	5–65 Minutes		1.5–3.5 Hours		4–5 Hours			
Test Garments	Micrograms per Hour[a]	Per Cent of Total	Micrograms per Hour[b]	Per Cent of Total	Micrograms per Hour[a]	Per Cent of Total	Micrograms per Hour[a]	Per Cent of Total
Respirator Pads	<20	—	<10[c]	—	<20	—	No sample	—
Gloves	2693	84	2218	87	557	81	435	60
Sleeves	417	13	288	11	75	11	205	28
T-Shirt	97	3	56	2	55	8	90	12
Total	3207	100	2562	100	687	100	730	100

[a] Based on a total of 14 garments on two men.
[b] Based on a total of seven garments on one man; exposure time, 2 hours.
[c] Experimental limit of analytical procedure was 20 micrograms when the sampling period was 1 hour and 10 micrograms when the sampling period was 2 hours.

for methyl parathion is the fact that, as described by Gunther & Blinn (1955), respiration of a leaf provides excellent opportunity for the co-distillation of steam-volatile insecticides, including those with comparatively low vapor pressures. In this connection, the high concentration of aromatic petroleum distillate in the spray formulations used in the Delta may be of importance.

This rapid decay rate of methyl parathion residues as determined by chemical studies agrees well with entomological observations. Furr & Lloyd (1957) reported that methyl parathion applied at the rate of 0.25 pound per acre on cotton lost its insecticidal effectiveness for boll weevils within 6 hours.

The results of the study of exposure of checkers to methyl parathion are shown in table 4. Right and left garments were both included. The gloves accounted for the majority of the residue recovered. The cotton sleeves and the T-shirts picked up lesser amounts. No detectable quantity was found on the respirator pads. The relative constancy of the per cent of total recovery for each test garment irrespective of the age of the residue was striking.

The total weight of methyl parathion residue obtained by all the test garments of the average checker was about 3 milligrams per hour in the first hour after spraying and less than 1 milligram per hour 4 hours after spraying. It is felt that the checker's opening of the squares with his fingers probably accounts for the majority of his exposure.

It was of interest to compare the decrease of residues on the test garments with the decay curves. The residues deposited on the test garments during the fifth hour were 79% less than during the first hour, comparing closely with the 78 and 88% decay of leaf residues in trials I and II for comparable times. A similar correlation was observed between exposure and residue decay for 4 and 24 hours.

Blood cholinesterase tests on these checkers performed 24 hours after the measured exposures showed no significant change from pre-exposure levels.

The rapid decay of methyl parathion and its consequent low residual toxicity were further supported by two observations. One grower allowed his children and calves free access to a cotton field which was treated repeatedly with 1.5% methyl parathion dust. Neither children nor livestock showed any ill effects from this exposure. The second incident involved 35 head of cattle which escaped from fencing and during the night ate the tops out of about 2 acres of cotton covered with the residue from five methyl parathion applications. The freshest residue was only 36 to 48 hours old. None of these cattle showed any harmful effects.

The residue decay curves for Guthion are shown in

Table 5.—Exposure of various body parts of a checker to recent application of guthion spray or dust to cotton.

	Spray (0.5 Lb./Acre)				Dust (0.25 Lb./Acre)			
	Age of Residue							
	5–35 Minutes		4–4.5 Hours		0–60 Minutes on Dew-Wet Leaves		4–5 Hours on Dry Leaves	
Test Garments	Micrograms per Hour[a]	Per Cent of Total	Micrograms per Hour[a]	Per Cent of Total	Micrograms per Hour	Per Cent of Total	Micrograms per Hour	Per Cent of Total
Respirator Pads	<40[b]	—	<40	—	<20	—	23	—
Gloves	5632	73	3910	74	10,720	58	>4072[c]	70
Sleeves	1584	20	946	18	4,450	24	717	12
T-Shirt	520	7	418	8	3,213	18	>1043[d]	18
Total	7756	100	5274	100	18,383	100	>5855	100

[a] Based on 30-minute exposure.
[b] Experimental limit of analytical procedure was 20 micrograms.
[c] Visible loss of dust from one glove during removal.
[d] Part of sample was lost during analysis.

figure 2. Although the application rate for the spray was twice that for the dust, previous treatment of the dust field had left a sufficient residue (about 5 micrograms/sq. in.) so that the application studied built up a total residue approximately equal to that from spraying. The decay rate of Guthion on cotton leaves was relatively slow. Its half-life was about 2 to 4 days.

In the test with the spray, traces of rain on the fifth and sixth days appeared to remove part of the residue. The slight rise in spray residue for the seventh day was associated with observed drift contamination from adjacent plots. Entomological experience with Guthion also paralleled chemical decay studies (Furr & Lloyd 1957).

One spot check in another field sprayed by airplane with 0.25 pound Guthion per acre showed 1.4 micrograms/sq.in. of leaf on the sixth day of a 7-day cycle.

The results of the exposure studies with Guthion are shown in table 5. The gloves accounted for the majority of the contamination. The sleeves and T-shirts picked up lesser amounts. A detectable amount was found on only one respirator pad. As was true for methyl parathion, the per cent of total recovery for each test garment was relatively constant regardless of the age of the residue.

For Guthion spray, the exposure represented by all the test garments was calculated to be about 8 mg. per hour in the first hour after spraying and about 5 mg. per hour in the fifth hour. The checkers' test garments picked up from the fresh dew-wet residues about 18 mg. of Guthion in the first hour. Four hours later, when the leaves had dried, their test garments retained only about 6 mg. per hour. No effect of this exposure on blood cholinesterase levels was found.

References Cited

Averell, P. R., and M. V. Norris. 1948. Estimation of small amounts of *O,O*-dimethyl *O,p*-nitrophenyl thiophosphate. Analyt. Chem. **20:** 753.

Batchelor, G. S., and K. C. Walker. 1954. Health hazards involved in the use of parathion in fruit orchards of North Central Washington. A.M.A. Arch. Indust. Hyg. **10:** 522.

Batchelor, G. S., K. C. Walker, and J. W. Elliott. 1956. Dinitroorthocresol exposure from apple-thinning sprays. A.M.A. Arch. Indust. Health **13:** 593.

Braid, P. E., J. P. Windish, and C. R. Ross. 1955a. Health hazards of drifting parathion dust cloud. A.M.A. Arch. Indust. Health **11:** 403.

Braid, P. E., J. P. Windish, and C. R. Ross. 1955b. Parathion spray concentrations and residues in Quebec apple orchards. A.M.A. Arch. Indust. Health **11:** 408.

Fahey, J. E., D. W. Hamilton, and R. W. Rings. 1952. Longevity of parathion and related insecticides in spray residues. Jour. Econ. Ent. **45:** 700.

Fowler, R. E. L. 1953. Manifestations of cottonfield insecticides in the Mississippi Delta. Jour. Agric. and Food Chem. **1:** 469.

Furr, R. E., and F. P. Lloyd. 1957. Preliminary field cage studies of the residual toxicity of methyl parathion, Guthion, and malathion to adult boll weevils. Presented at Entomological Society of America Meeting. Memphis, Tenn. on Dec. 5. (Abstract in Bull. Ent. Soc. America **3:** 45.)

Gunther, F. A., and R. C. Blinn. 1955. Analysis of insecticides and acaracides. Interscience Publishers, New York, p. 132.

Hayes, W. J., Jr., E. M. Dixon, G. S. Batchelor, and W. M. Upholt. 1957. Exposure to organic phosphorus sprays and occurrence of selected symptoms. Publ. Health Repts. **72:** 787.

Limperos, G., and K. E. Ranta. 1953. A rapid screening test for the determination of the approximate cholinesterase activity of human blood. Science **117:** 453.

Michel, H. O. 1949. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. Jour. Lab. and Clin. Med. **34:** 1564.

Quinby, G. E., and A. B. Lemmon. 1958. Parathion residues as a cause of poisoning in crop workers. Jour. Amer. Med. Assoc. **166**(7): 740.

Roussel, J. S., and D. Clower. 1955. Resistance to the chlorinated hydrocarbon insecticides in the boll weevil. Louisiana Agric. Expt. Sta. Circ. **41.**

Sumerford, W. T., W. J. Hayes, Jr., J. M. Johnston, K. Walker, and J. Spillane. 1953. Cholinesterase response and symptomatology from exposure to organic phosphorus insecticides. A.M.A. Arch. Indust. Hyg. and Occup. Med. **7:** 383.

Upholt, W. M., G. E. Quinby, G. S. Batchelor, and J. P. Thompson. 1956. Visual effects accompanying TEPP induced miosis. A.M.A. Arch. Ophthalmol. **56:** 129.

Further Studies on the Metabolism of Thimet by Plants, Insects, and Mammals[1]

J. S. Bowman[2] and J. E. Casida,[3] *Department of Entomology, University of Wisconsin, Madison*

Abstract

The oxidative and hydrolytic metabolism of Thimet by plants, insects and mammals were further studied with chromatographic and radiotracer techniques. Bean plants, southern armyworms (*Prodenia eridania* (Cram.)), albino rats, and a cow were utilized. The proportions of Thimet, oxidized derivatives, and hydrolysis products were determined with bean plants which had absorbed Thimet through their roots, with armyworms which had fed on these plants, and with the feces of the armyworms. Armyworms were more efficient than bean plants *in vivo* in oxidation of the phosphorothioate group of Thimet and in hydrolysis of the oxidation products. With the mammals, the excretory products and tissue residues were investigated. Extreme difficulty was encountered in extraction of the radioactivity from the tissues of the treated rats and cow. The relative insect and mammalian toxicity and stability to hydrolysis by alkali are reported for Thimet and its oxidation products.

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by research grants from the American Cyanamid Co. and the U. S. Atomic Energy Commission (Contract No. AT(11-1), Project No. 14). Accepted for publication June 13, 1958.

[2] Present Address: Hazleton Laboratories, Inc., Falls Church, Virginia.

[3] The authors wish to thank Dr. R. W. Young of the American Cyanamid Company for supplying certain of the chemicals used in this study.

Thimet (*O,O*-diethyl S-ethylthiomethyl phosphorodithioate), when absorbed by plants, is first oxidized at the thioether sulfur to form the phosphorodithioate sulfoxide and sulfone (*O,O*-diethyl S-ethylsulfinylmethyl phosphorodithioate and *O,O*-diethyl S-ethylsulfonylmethyl phosphorodithioate) and is then oxidized at the thiono sulfur to form the phosphorothiolate sulfoxide and sulfone (*O,O*-diethyl S-ethylsulfinylmethyl phosphorothiolate and *O,O*-diethyl S-ethylsulfonylmethyl phosphorothiolate). These oxidation products are all more potent anticholinesterase agents than the Thimet precursor and persist in plants for relatively long periods of time (Bowman & Casida 1957, Metcalf *et al.* 1957).

The *in vivo* oxidation and hydrolysis of Thimet was further studied with plants, insects and mammals.

METHODS AND RESULTS.—*Synthesis of P-32 O,O-diethyl S-ethylthiomethyl phosphorodithioate (Thimet).*—Three and four-tenths millimoles phosphorus-32 pentasulfide (prepared by isotope exchange according to Casida 1959) was suspended in 2.0 ml. benzene in a small reaction flask fitted with a dropping funnel and a reflux condenser. The reaction flask was placed in a 60° C. water bath, and the contents were stirred with a magnetic bar while 15 millimoles anhydrous ethanol in 2.0 ml. benzene were added dropwise. After rinsing the dropping funnel with an additional ml. of benzene, the reaction mixture was refluxed for 2 hours, cooled to room temperature, and 6.8 millimoles potassium hydroxide, dissolved in 1.8 ml. ethanol and 1.4 ml. benzene, were added dropwise. The reflux condenser was then replaced with a distillation condenser, and the mixture was distilled with the addition of further benzene through the dropping funnel to make up for volume loss until the temperature of the distilling vapors reached 80° C. The benzene was removed by distillation with a water bath, the residue dried with air and 15 ml. acetone were added. Eight and nine-tenths millimoles of ethylthiomethyl chloride were added dropwise, and the dropping funnel was washed with 1.0 ml. acetone. The reaction was stirred at room temperature for 12 hours. The potassium chloride which formed was filtered off, washed with acetone, dried and weighed. After stripping the acetone from the filtrate, the residue was chromatographed on a celite-isooctane-methanol column (Bowman & Casida 1957), and the products were identified by infrared spectra (10% solutions in chloroform) and by the coincidence of the rate of elution of the total phosphorus and radioactive phosphorus from the column. Four and eight-tenths millimoles potassium chloride, 4.6 millimoles of *O,O*-diethyl S-ethylthiomethyl phosphorodithioate, and 0.2 millimole of *O,O*-diethyl S-ethylthiomethyl phosphorothiolate were recovered. Therefore, the over-all yield from red phosphorous to Thimet was about 70%, and the product had a specific activity of 18 millicuries per gram.

Toxicity and Stability to Hydrolyis by Alakali of Thimet and Its Oxidative Derivatives.—Approximate rat LD_{50}'s were determined with 150-gram male albino rats 24 hours after subcutaneous administration of the Thimet derivatives in corn oil solutions. These oxidative derivatives were synthesized in the Stamford Laboratories of th- American Cyanamid Company, purified on celite-isoe octane-methanol columns, and characterized by infrared spectra according to Bowman & Casida (1957). Under these assay conditions, Thimet *per se* was the least toxic, and *O,O*-diethyl S-ethylsulfonylmethyl phosphorothiolate and *O,O*-diethyl S-ethylthiomethyl phosphorothiolate were the most toxic (table 1).

The relative toxicity to house flies was evaluated by topical application of acetone solutions of the various derivatives to the abdomens of female house flies (*Musca domestica* L.) (method of Krueger & Casida 1957). Thimet *per se* was among the most toxic of the derivatives to house flies, while the order of toxicity of the oxidation products was similar for the house flies and rats (table 1).

Alkaline hydrolysis was studied by dissolving 25 mg. of each compound and 25 mg. of Triton X-155 emulsifier in 5 ml. 95% ethanol, to which was then added 45 ml. of freshly prepared 0.1 M. aqueous sodium carbonate. After various periods of incubation at 28° C., 3.0 ml. aliquots of the reaction mixture were partitioned with equal volumes of chloroform, and the percentage recovery in each phase was determined by colorimetric analyses for total phosphorus (Allen 1940). When the logarithm of the percentage of phosphorus recovered in the chloroform phase was plotted against time, a linear relationship up to 95% by analysis resulted. Therefore, the results in table 1 are expressed as first order hydrolysis constants. The phosphorothiolates were 22 to 60 times more rapidly hydrolyzed than the phosphorodithioates, and the sulfoxides and sulfones were 42 to 136 times more rapidly hydrolyzed than the sulfides under these assay conditions.

Studies on Thimet-P-^{32} Metabolism by Rats.—Two groups of 10 male albino rats each were treated orally with Thimet-P^{32} in corn oil. One group received a single dose of 2.0 mg./kg. and the other received six daily doses of 1.0 mg./kg. The rats were held in metabolism cages (Comar 1955) and the excreta analyzed for total radioactivity. Rats treated with the single dose of 2.0 mg./kg. excreted 35% of the administered radioactivity in the urine and 3.5% in the feces within 144 hours. Rats treated with six daily doses of 1.0 mg./kg. excreted only 12% of the total administered radioactivity in the urine and 6% in the feces within 7 days. On the sixth and seventh days equal total amounts of Thimet metabolites were excreted in the urine and feces. The hydrolysis products appearing in the urine of rats within the first 2 days from daily oral doses of 1.0 mg./kg. were sep-

Table 1.—Biological activity and stability to hydrolysis by alkali of Thimet and its oxidation products.

COMPOUND	LD_{50} as MG./KG.		HYDROLYSIS CONSTANT (MIN.$^{-1}$)
(R = C_2H_5)	Rat—♂	House Fly—♀	0.1 M Na_2CO_3
$(RO)_2P(S)SCH_2SR$	8 –10	1.5	0.00011
$(RO)_2P(S)SCH_2S(O)R$	2 – 4	5.5	0.013
$(RO)_2P(S)SCH_2S(O_2)R$	1.8– 2.0	4.5	0.015
$(RO)_2P(O)SCH_2SR$	0.6– 0.8	1.1	0.0066
$(RO)_2P(O)SCH_2S(O)R$	1.4– 1.6	5.5	0.28
$(RO)_2P(O)SCH_2S(O_2)R$	0.6– 0.8	1.5	0.35

arated by ion-exchange chromatography (Plapp & Casida 1958) and found to consist of 17% *O,O*-diethyl phosphoric acid, 80% *O,O*-diethyl phosphorothioic acid, and 3% *O,O*-diethyl phosphorodithioic acid.

Extreme difficulty was encountered in extracting radioactive metabolites of Thimet from the rat tissues. From the group of rats treated with 1.0 mg./kg. Thimet daily for 6 days, the liver, kidney, and brain tissues were removed and fractionated. These tissues were minced, extracted with hot water and then with acetone. The dry acetone-powers contained an average of 87% of the initial radioactivity from the tissues. Of the radioactivity which was extracted from the tissues, only about 39% extracted into chloroform from water. Another group of rats were treated orally with 16 mg./kg. Thimet-P^{32}, and at the time of death (0.5 to 5.5 hours), tissue samples of liver, kidney, heart, fat, brain, and muscle were removed for analysis. When the samples were extracted first with chloroform and then with acetone, 69% of the tissue radioactivity remained in the acetone-powders. About 61% of the radioactivity which was removed from the tissues was extractable into chloroform from water. Another group of rats was treated orally with 2.0 mg./kg. Thimet-P^{32} and sacrificed after 2 hours to obtain liver samples. These samples were fractionated with trichloroacetic acid (Jandorf & McNamara 1950) and with the hot water and acetone system described, but again 67% and 55%, respectively, of the tissue radioactivity remained in the insoluble residue. The nature of the Thimet derivatives in the unextractable portion from rat tissues is not known. It appears improbable that Thimet was degraded to phosphoric acid and the radioactivity resynthesized into tissue proteins because of the short metabolism times involved (as low as 0.5 hour) and the failure to recover any radioactive phosphoric acid in the rat urine even after longer intervals. It is unlikely that Thimet *per se* was bound on the tissue to this extent since Thimet added to tissue could be readily recovered with very little binding. Possibly the radioactivity from Thimet was present as diethyl phosphorylated protein.

An attempt was made to quantitate rat liver slice preparations (DuBois *et al.* 1950) for studying the oxidative metabolism of Thimet. Three μgm. Thimet-P^{32} emulsified in 3.0 ml. of a glucose-containing phosphate buffer at pH 7.0 were incubated with 300 mg. fresh liver slices at 37° C. in a 100% oxygen atmosphere for times of 5 and 30 minutes and 1, 2, 4, and 8 hours. The total radioactivity was recovered by washing the flasks and liver slices with water, acetone and chloroform. The chloroform-soluble radioactivity was run on celite-isooctane-methanol columns to determine the nature of any oxidative metabolites formed. Less than 1% of the radioactivity appeared as hydrolysis products or unextractable residue from the proteins with any of the incubation times. Up to 10% of the phosphorodithioate sulfoxides and sulfones and less than 1% of the phosphorothiolate sulfoxides and sulfones were formed by the liver slices. It is possible that lower levels of Thimet than the 1,000 p.p.m. used would yield a higher proportion of oxidation and hydrolysis products.

Oral Administration of a Toxic Dose of Thimet-P^{32} to a Cow.—Radioactive Thimet-P^{32} was formulated in gelatin capsules and administered orally to a lactating Holstein cow at 3.04 mg./kg. (1.1×10^9 c.p.m. for total dose). This proved to be a highly toxic dose for the cow. When acute symptoms developed, the cow was treated intramuscularly with atropine sulfate injections. At periodic intervals up to 96 hours after treatment, samples of milk, urine, feces, jugular blood, and fat biopsies were taken and assayed for total radioactivity. The cholinesterase activity of the whole blood samples was determined (Casida *et al.* 1958), the nature of the hydrolysis products in the urine was evaluated by ion-exchange chromatography, and the per cent of the radioactivity in the milk, blood, urine, and feces, which could be extracted into chloroform, was determined. Records were kept on the total output of urine, feces, and milk on the catheterized cow. The cow was sacrificed 4 days after treatment, and samples of various tissues were collected, fractionated and assayed for total radioactivity.

Of the administered radioactivity, 59% was excrete in the urine within 72 hours after treatment, the cumulative per cent excreted being linear with time for this 3-day period. Partitions of each sample between chloroform and water gave complete recovery of the radioactivity in the aqueous phase, indicating that only hydrolysis products of Thimet were present in the urine. The initial hydrolysis product excreted in the urine was *O,O*-diethyl phosphorothioic acid (fig. 1), and this metabolite steadily declined with time as *O,O*-diethyl phosphoric acid increased (fig. 1). Diethyl phosphoric acid was the major hydrolyzed metabolite in the urine during the final 60 hours the cow was kept alive with atropine. Excretion of *O,O*-diethyl phosphorodithioic acid in the urine was uniformly low throughout the experiment. Analysis of the bile fluid taken at the time the cow was sacrificed revealed the same proportion of the various hydrolysis products as the final sample of urine taken just prior to death of the cow. However, this observation is probably only a coincidence since the bile fluid is not directly related in function to urinary excretion.

Of the total administered radioactivity, 0.8% was excreted in the feces by 96 hours after treatment. Each feces sample was fractionated by extracting three times

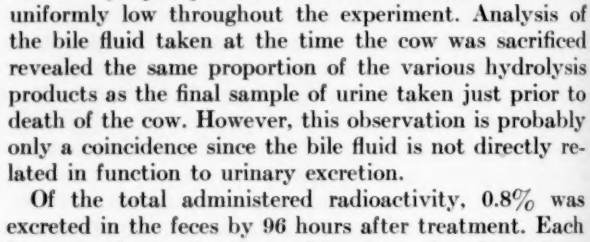


FIG. 1.—Proportions of Thimet hydrolysis products excreted in the urine by a Holstein cow treated orally at 3.04 mg. per kg.

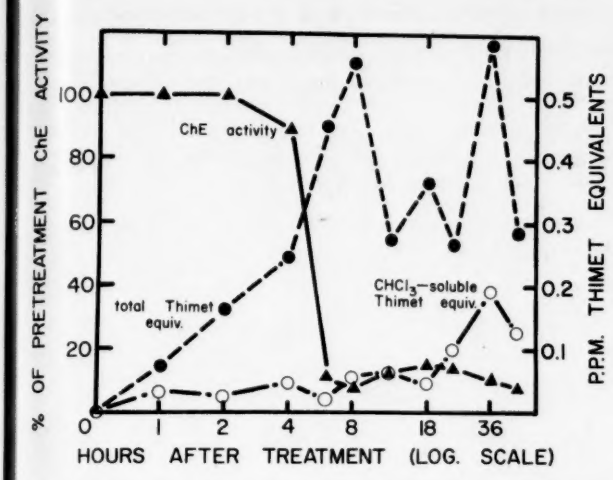


FIG. 2.—Blood cholinesterase activity and levels of Thimet equivalents in the blood after oral administration at 3.04 mg. per kg.

with an acetone-water mixture which was filtered through cheese cloth. The acetone was evaporated from the pooled extracts, and the resulting water residue was partitioned with chloroform. The chloroform-soluble materials were partitioned between hexane and acetone and water (5:4:7) by the method of Bowman & Casida (1957). Of the total radioactivity in the feces, 20% appeared as chloroform-soluble materials 6 hours after treatment, and this diminished to 10% at 12 hours and 10% at 24, 48, and 96 hours after treatment. The partitioning properties of these chloroform solubles indicated that the initial oxidative metabolites appearing in the feces were *0,0*-diethyl S-ethylsulfinylmethyl phosphorodithioate and/or *O,O*-diethyl S-ethylsulfonylmethyl phosphorodithioate; whereas, later in the experimental period a higher proportion of unoxidized Thimet was excreted.

Samples of jugular blood taken at various intervals after treatment were assayed for the per cent of pretreatment cholinesterase activity, the total radioactive materials present, and the proportion of chloroform-soluble metabolites (fig. 2). There was a steady increase in the total p.p.m. of Thimet equivalents in the blood during the first 6 to 8 hours after treatment, at which time the cholinesterase-level had reached a minimum. Also, at 6 to 8 hours after treatment the cow developed severe symptoms of phosphate poisoning, including an increased rate of respiration, salivation and extreme muscular tremors. At 8 hours after treatment there was a complete loss of coordination. Atropine sulfate was administered intramuscularly at the rate of 13 to 26 mg. per injection for a total of 78 mg. to keep the cow atropinized during the duration of the experiment. The atropine quickly relieved the symptoms so that at 15 hours after the administration of Thimet the animal was again sufficiently coordinated to stand. This may account for the drop in total Thimet-equivalents in the blood between 12 and 24 hours after treatment and the slight rise in cholinesterase activity during this period. There was a slight increase in the proportion of chloroform-soluble metabolites in the blood between 18 and 36 hours after treatment.

Table 2.—Tissue residues 96 hours after oral administration of Thimet-P^{32} to a Holstein cow at 3.04 mg./kg.

TISSUE	TOTAL P.P.M. OF THIMET EQUIVALENTS	P.P.M. THIMET EQUIVALENTS EXTRACTABLE WITH[a]	
		Chloroform	Acetone
Liver	10.3	0.5	0.5
Kidney	5.2	0.8	0.5
Lung	1.5	0.2	0.1
Heart	0.64	0.59	0.02
Muscle (loin)	0.29	0.00	0.02
Brain	0.24	0.05	0.02
Fat (mesentery)	0.03	0.00	0.00

[a] Remainder of radioactivity in the acetone-powders of the tissues.

Milk samples were taken at 8, 24, 32, and 56 hours after treatment, and the p.p.m. of total Thimet equivalents were found to be 0.07, 0.25, 0.30, and 0.53, respectively. Fat biopsies showed 0.05 p.p.m. total Thimet equivalents at 4 hours and 0.06 p.p.m. at 24 hours after treatment. The radioactivity in the milk and fat biopsy samples was too low for fractionation.

Upon sacrifice of the cow 96 hours after treatment, the radioactive residues in various tissues were determined. The radioactivity in several tissues was fractionated (table 2), and with the other tissues the total radioactivity was expressed as Thimet-equivalents (table 3). The extraction was accomplished by blending 50 grams of each tissue with 200 ml. chloroform in a Waring blender, and after centrifugation, the residue was reextracted with 200 ml. acetone. The acetone was evaporated, the aqueous residue taken up in chloroform and dried with anhydrous sodium sulfate. Most of the radioactivity with all the tissues remained in the dry acetone-powders from the tissues and very little could be extracted with either chloroform or acetone. The highest total tissue residues appeared in the liver, kidney, lung, alimentary canal and glandular tissues. Fat samples from many sites in the body were very low in Thimet metabolites.

Table 3.—Total Thimet equivalents in various tissues 96 hours after oral administration of Thimet-P^{32} to a Holstein cow at 3.04 mg/kg.

P.P.M. THIMET EQUIVALENTS (RANGE)	TISSUES (IN DECREASING ORDER)
2.78–1.04	Rumen wall, omasum wall, reticulum wall, parotid gland, submaxillary gland, gall bladder, adrenal gland, rib bone, subiliac lymph node;
0.89–0.52	Submaxillary lymph node, small intestine wall, appendix wall, intestine wall near rectum, abdominal wall, pancreas, spleen, tongue, supramammary lymph node;
0.48–0.11	Dorsi muscle, urinary bladder, mammary gland, placenta, leg muscle, thyroid gland, back muscle, small intestinal fat, sheath of spinal cord, hide;
0.06–0.02	Spinal cord, abdominal fat, large intestinal wall, large intestinal fat, subcutaneous fat, kidney fat.

Table 4.—The conversion of Thimet to its oxidation and hydrolysis products by bean plants and the subsequent fate of these metabolites when ingested by southern armyworm larvae.

Metabolite (R = C_2H_5)	Per Cent of Total Extractable Radioactivity as Following Metabolites								
	1 Day			4 Days			12 Days		
	Plant	Insect	Feces	Plant	Insect	Feces	Plant	Insect	Feces
Non-hydrolyzed									
$(RO)_2P(S)SCH_2SR$	2.8	0.9	6.9	0.4	0.6	0.2	0.5	0.2	1.2
$(RO)_2P(O)SCH_2SR$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$(RO)_2P(S)SCH_2S(O)R$ and/or $(RO)_2P(S)SCH_2S(O_2)R$	76.2	3.4	5.4	81.1	3.7	3.5	64.0	3.1	3.3
$(RO)_2P(O)SCH_2S(O)R$ and/or $(RO)_2P(O)SCH_2S(O_2)R$	0.0	16.7	16.7	0.5	8.7	8.3	8.5	10.8	10.5
Hydrolyzed									
H_3PO_4	0.6	3.6	5.3	2.6	7.7	0.0	1.6	0.4	0.0
$(RO)_2P(O)OH$	1.7	40.5	41.5	4.0	42.8	43.5	8.4	45.2	28.6
$(RO)_2P(S)OH$	8.1	25.0	21.7	9.2	35.7	43.6	12.7	39.0	54.0
$(RO)_2P(S)SH$	7.6	6.8	2.6	1.5	0.8	0.9	3.0	1.3	2.4
Unknown	3.0	3.1	0.0	0.7	0.0	0.0	1.3	0.0	0.0

Metabolism of Thimet by Bean Plants and Armyworms Feeding on the Plants.—Red kidney bean plants, with their first trifoliate leaves, were placed in an emulsion of 500 p.p.m. Thimet-P^{32} for 1 day, after which the roots were washed and the plants were transplanted into white sand. At 1, 4, and 12 days after the introduction of Thimet into the plants, the primary and secondary leaves from 25 plants were homogenized with 100 ml. chloroform and 20 ml. water in a Waring blender. At these same time intervals, 50 third- or fourth-instar southern armyworm larvae (*Prodenia eridania* (Cram.)) were allowed to feed on similar groups of treated plants for 48 hours, and they were then homogenized in the same way. The fecal droppings from the armyworms were collected and extracted by grinding with a mixture of chloroform and water in a mortar. The chloroform-soluble materials from the plants, insects and insect feces were chromatographed on celite-isooctane-methanol columns, and the peaks were characterized by their elution positions and partitioning properties between hexane, acetone and water (5:4:7). The radioactive hydrolysis products were chromatographed on anion exchange columns with carrier *O,O*-diethyl phosphoric, phosphorothioic, and phosphorodithioic acids, and the elution positions were determined by colorimetric total phosphorus analysis.

The major non-hydrolyzed metabolites of Thimet in the bean plants throughout the experimental period were *O,O*-diethyl S-ethylsulfinylmethyl and/or S-ethylsulfonylmethyl phosphorodithioate (table 4). Incomplete separation on the columns of the sulfoxide from the sulfone metabolites did not allow distinct consideration of these materials. In the insect and insect feces, the major non-hydrolyzed metabolites were *O,O*-diethyl S-ethylsulfinylmethyl and/or S-ethylsulfonylmethyl phosphorothiolates. The per cent of non-hydrolyzed materials as Thimet was low relative to the oxidation products, and no *O,O*-diethyl S-ethylthiomethyl phosphorothiolate was detected in the plant, insect or feces. In the plant, the oxidation of the thioether grouping occurred more readily than the phosphorothioate grouping. The insect, however, was more efficient than the plant in phosphorothioate oxidation.

Of the hydrolyzed metabolites of Thimet, *O,O*-diethyl phosphorothioic acid predominated in the plants, but *O,O*-diethyl phosphoric acid increased with time. In the insect and insect feces, *O,O*-diethyl phosphoric acid was most important early, but *O,O*-diethyl phosphorothioic acid increased proportionately with time. Phosphoric and *O,O*-diethyl phosphorodithioic acids were also found in plant, insect, and feces. The "unknown" hydrolysis product of Thimet, which eluted from the ion-exchange column between diethyl phosphoric and diethyl phosphorothioic acids (pH 2 HCl plus methanol to pH 1 HCl plus methanol elution gradient), may be a deethylated derivative of Thimet or of its oxidation products. It was found in the plants at 1, 4, and 12 days after the introduction of the Thimet source but only appeared in the insects feeding on the plants 1 day after treatment and did not appear in the feces. The insect was much more efficient than the plant in hydrolysis of the Thimet oxidation products.

References Cited

Allen, R. J. L. 1940. The estimation of phosphorus. Biochem. Jour. **34**: 345–7.

Bowman, J. S., and J. E. Casida. 1957. Metabolism of the systemic insecticide *O,O*-diethyl S-ethylthiomethyl phosphorodithioate (Thimet) in plants. Jour. Agric. and Food Chem. **5**: 192–7.

Casida, J. E. 1959. Isotype exchange for synthesis of phosphorothioate insecticides. (In press)

Casida, J. E., P. E. Gatterdam, J. B. Knaak, R. D. Lance, and R. P. Neidermeier. 1958. Bovine metabolism of organophosphate insecticides. I. Subacute feeding

studies with *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Phosdrin insecticide). Jour. Agric. and Food Chem. **6**: 658–62.

Comar, D. L. 1955. *Radioisotopes in Biology and Agriculture.* McGraw-Hill, New York.

DuBois, K. P., J. Doull, and J. M. Coon. 1950. Studies on the toxicity and pharmacological action of octamethylpyrophosphoramide (OMPA: Pestox III). Jour. Pharmacol. and Exptl. Therap. **99**: 376–93.

Jandorf, B. J., and P. D. McNamara. 1950. Distribution of radiophosphorus in rabbit tissues after injection of phosphorus-labeled diisopropyl fluorophosphate. Jour. Pharmacol. and Exptl. Therap. **98**: 77–84.

Krueger, H. R., and J. E. Casida. 1957. Toxicity of fifteen organophosphorus insecticides to several insect species and to rats. Jour. Econ. Ent. **50**: 356–8.

Metcalf, R. L., T. R. Fukuto, and R. B. March. 1957. Plant metabolism of dithiosystox and Thimet. Jour. Econ. Ent. **50**: 338–45.

Plapp, F. W., and J. E. Casida. 1958. Ion exchange chromatography for hydrolysis products of organophosphorus insecticides. Analyt. Chem. **30**: 1622–4.

Biological Effectiveness and Persistence of Malathion and Lindane Used for Protection of Stored Wheat[1]

F. A. GUNTHER, D. L. LINDGREN, and R. C. BLINN, *University of California Citrus Experiment Station, Riverside*

ABSTRACT

The biological effectiveness of various concentrations of malathion and of lindane used for control of adults of *Sitophilus granarius* (L.), *S. oryza* (L.), and *Rhyzopertha dominica* (F.) in stored wheat, and the residual half-life of these compounds in and on the grain, were determined. Malathion lost biological effectiveness with time and had a residual half-life of 5.6 months. Lindane remained unaffected both biologically and chemically throughout the storage period.

Protectant dusts and sprays incorporating an insecticide have recently been employed effectively to prevent insect infestation of stored grain. Lindgren *et al.* (1954) report that malathion was used effectively for this purpose, and Freeman (1957) reports that BHC has been used for protecting stored grain in several countries.

Because of the promising nature of the control of stored grain insects by these materials, it was of interest to determine by both chemical and bioassay procedures the residual behavior of malathion and lindane on and in grain under typical storage conditions.

MATERIALS AND METHODS.—Three-kilogram quantities of wheat of 12% moisture content were treated on July 14–15, 1954, with the amount of 1% malathion in wheat stock calculated to give 1, 2, 4, 8, and 16 p.p.m. of actual malathion on and in the grain. Each lot of wheat was mixed for 3 hours, and 200-gram aliquots were then placed in 1-pint Mason jars, sealed, and stored at 75° F. in darkness. A control lot of wheat was handled in the same manner.

Later, on April 6, 1956, 2½-kilogram quantities of wheat of 11.5% moisture content were treated with the amount of 1% lindane solution in ethylene dichloride calculated to give 4, 8, and 12 p.p.m. actual lindane on and in the grain. Each lot of wheat was mixed thoroughly, spread in a thin layer to allow complete evaporation of the ethylene dichloride, and then stored at 75° F. in the darkness in a sealed can. A control lot of wheat was handled in the same manner.

Bioassay.—The effectiveness of malathion was tested by periodically confining 100 adults each of the granary weevil, *Sitophilus granarius* (L.), the rice weevil, *S. oryza* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.) in 200-gram samples of treated grain at the time of treatment and then at monthly intervals after treatment. The insects were exposed for a period of 10 days and were then removed and checked for mortality.

The effectiveness of lindane was tested by periodically confining 100 adults each of these same insect species in 150-gram samples of treated grain at the time of treatment and then 3, 5½, 10, and 15 months after treatment. The insects were exposed for a period of 30 days and were then removed and checked for mortality.

[1] Paper No. 1053, University of California Citrus Experiment Station, Riverside, California. Accepted for publication June 16, 1958.

Table 1.—Effectiveness of malathion-treated wheat (12% moisture content) for control of adults of *Sitophilus granarius* (A), *S. oryza* (B), and *Rhyzopertha dominica* (C).

Months After Treatment	Per Cent Kill at Various Dosages														
	16 p.p.m.			8 p.p.m.			4 p.p.m.			2 p.p.m.			1 p.p.m.		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	100	100	99	100	100	98	100	100	37	98	96	11	32	48	4
1	100	100	62	100	100	63	41	79	9	2	1	0	3	1	1
2	100	100	35	100	100	45	8	25	—	—	—	—	—	—	—
3	100	100	—	100	100	—	—	—	—	—	—	—	—	—	—
4	99	100	—	100	100	—	—	—	—	—	—	—	—	—	—
5	89	97	—	100	97	—	—	—	—	—	—	—	—	—	—
6	30	44	—	46	81	—	—	—	—	—	—	—	—	—	—

Table 2.—Effectiveness of lindane-treated wheat (11.5% moisture content) for control of adults of *Sitophilus granarius* (A), *S. oryza* (B), and *Rhyzopertha dominica* (C).

Months After Treatment	Per Cent Kill at Various Dosages											
	12 p.p.m.			8 p.p.m.			4 p.p.m.			Control		
	A	B	C	A	B	C	A	B	C	A	B	C
0	100	100	100	100	100	100	100	100	100	35	[illegible]	5
3	100	100	100	100	100	100	100	100	100	29	42	3
5½	100	100	100	100	100	100	100	100	99	12	11	[illegible]
10	100	100	100	100	100	100	100	100	100	34	3	4
15	100	100	100	100	100	100	97	68	97	9	32	33

Table 3.—Residues of malathion on and in treated wheat (12% moisture content).

Months After Treatment	P.p.m. Found[a] at Various Dosages			
	16 p.p.m.	8 p.p.m.	4 p.p.m.	2 p.p.m.
0	7.1	6.5	1.3	0.7
1	4.0	4.7	1.2	0.3
2	3.3	4.3	1.0	0.3
3	3.5	4.2	0.9	0.7
4	3.6	3.0	0.7	0.1
5	2.9	2.8	0.8	0.7
6	2.1	1.9	0.9	0.7

[a] Corrected for background (0.3 p.p.m.) and for recovery (100%).

Table 4.—Residues of lindane on and in treated wheat (11.5% moisture content).

Months After Treatment	P.p.m. Found[a] at Various Dosages		
	12 p.p.m.	8 p.p.m.	4 p.p.m.
0	7.7	5.1	2.8
3	7.7	4.1	1.9
5½	7.1	4.3	1.9
11	—	4.8	2.3
15	7.4	4.0	2.3

[a] Corrected for background (0.0 p.p.m.) and recovery (90.1%).

Table 5.—Half-life values for malathion residues on and in wheat (12% moisture content).

Dosage (P.p.m.)	Half-Life Value, Months
16	6.0
8	5.0
4	5.5
2	6.5
Average	5.6

Chemical Assay.—Analysis for malathion content of treated grain was conducted at the time of the treatment and then at monthly intervals. Two hundred-gram samples were ground in a coffee grinder, processed with redistilled carbon tetrachloride in the manner and with the equipment previously described (Gunther & Blinn 1955) to afford final stripping solutions. Aliquots of the stripping solutions were assayed for malathion by a colorimetric procedure (Norris *et al.* 1954).

Analysis for lindane content of treated grain was conducted at the time of the initial treatment and then at 3, 5½, 11, and 15 months after treatment. Three hundred and fifty-gram samples were ground in a coffee grinder, then extracted with methylene chloride in a modified Soxhlet-type extractor for 6 hours. Aliquots of this solution were concentrated in a Kuderna-Danish evaporative concentrator to about 5 ml. To this was added glacial acetic acid, and the volume reduced to half by careful distillation of excess solvents through a distilling column. This solution was assayed for lindane by a colorimetric procedure (Schechter & Hornstein 1952).

Results and Conclusions.—Results of the bioassay tests of malathion-treated grain are presented in table 1; those of lindane-treated grain are presented in table 2.

Results of the chemical analyses of malathion-treated grain are presented in table 3, and those of lindane-treated grain in table 4. The residue values for malathion in the 1-p.p.m. lot of grain were not included as the values were of borderline sensitivity with the assay method used.

It is evident from the data of both the bioassay and the chemical assay that lindane was stable under the conditions of storage utilized in these studies, whereas malathion was lost. Half-life values (Gunther & Blinn 1955) for malathion residues on and in the wheat are shown in table 5. These half-life values are derived from the plot of the logarithm of the residue value against elapsed time after treatment.

References Cited

Freeman, J. A. 1957. Infestation of grain in international trade. A review of problems and control methods. Jour. Sci. Food Agric. **8**(11): 623–9.

Gunther, F. A., and R. C. Blinn. 1955. Analysis of insecticides and acaricides. Interscience Publishers, New York-London.

Lindgren, D. L., H. E. Krohne, and L. E. Vincent. 1954. Malathion and Chlorthion for control of insects infesting stored grain. Jour. Econ. Ent. **47**(4): 705–6.

Norris, M. V., W. A. Vail, and P. R. Averell. 1954. Colorimetric estimation of malathion residues. Jour. Agric. Food Chem. **2**(11): 570–3.

Schechter, M. S., and I. Hornstein. 1952. Colorimetric determination of benzene hexachloride. Analyt. Chem. **24**(3): 544–8.

Influence of Black Fly Control on the Incidence of *Leucocytozoon* Disease in South Carolina Turkeys[1,2]

DARRELL W. ANTHONY, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*, and
DALE J. RICHEY, *South Carolina Agricultural Experiment Station, Clemson*

ABSTRACT

Experiments were conducted in Jasper County, S. C., to determine if the control of black flies (*Simulium*), vectors of *Leucocytozoon smithi* (Lavern & Lucet), would reduce or eradicate this disease in turkeys. Plots of approximately 11 and 4 square miles, each containing a turkey farm, were treated by aircraft with 5% DDT-oil solution at the rate of 0.033 pound of insecticide per acre to destroy black flies breeding in the streams. In the large plot good control of larvae and pupae was obtained for 4 weeks, and dry weather practically eliminated breeding during the following 8 weeks. Observations of turkey poults placed on pasture 4 weeks after treatment failed to reveal black flies, but *Leucocytozoon* infections were evident after 2 weeks' exposure. Other blood-sucking Diptera collected from the turkeys included *Culicoides* spp., *Aedes* spp., *Diachlorus ferrugatus* (F.), and *Chrysops univittata* Macq. Good control of larvae was also obtained for 4 weeks in the small plot, and dry weather kept breeding at a low level thereafter. However, pupae of *S. slossonae* D. & S. and *congareenarum* (D. & S.) were collected nearly every week of the study, and a few adults may have been available to attack the turkeys. Leucocytozoonosis was evident after the turkeys were exposed 2 weeks on the pasture.

Extensive studies have been made to determine the insects responsible for the transmission to turkeys of the protozoan parasite *Leucocytozoon smithi* (Lavern & Lucet). The disease has been reported from most of the southern States and from a few midwestern and northern States, but the vectors have been demonstrated in only a few instances. Skidmore (1933) showed the black fly, *Simulium occidentale* Townsend, to be a vector in Nebraska, and Johnson *et al.* (1938) found *S. jenningsi* Mall. (=*nigroparvum* (Twinn)) capable of transmitting the disease in Virginia. Underhill (1939), also working in Virginia, reported another species, *S. slossonae* D. & S., as a pest of turkeys. Recent studies by Jones & Richey (1956) showed that *S. slossonae* and *congareenarum* (D. & S.) were pests of turkeys in South Carolina, and confirmed an earlier report (U. S. Bureau of Animal Industry 1953) that *slossonae* is an important vector of this disease.

Black flies of the genus *Simulium* breed only in freshwater streams, principally in their swift-flowing areas. Since these flies are the only known vectors of *L. smithi*, it was thought that insecticide applications to the streams in the vicinity of turkey farms might reduce disease incidence. Two turkey farms in Jasper County, South Carolina, with numerous black fly breeding sites near the poultry ranges, were therefore selected for such studies.

METHODS.—Black fly breeding in most of the freshwater streams in the county presented the possibility of adult flies from untreated streams infiltrating the treated areas. One of the test plots was therefore much larger than the other in order to determine the size of area to treat to prevent flies from reaching the turkeys from untreated sources. The turkey pastures were near the center of each plot. The large plot covered about 11 square miles, and the closest untreated black fly breeding area was about 8½ air miles from the poultry pastures. The small plot covered about 4 square miles, and the closest untreated breeding area was about 2½ air miles away.

The plots were sprayed by aircraft during the first 2 weeks in March 1955. A 5% DDT-oil solution was applied to both plots at the rate of 0.033 pound of DDT per acre on a total-acre basis, or 0.2 pound per acre sprayed. The spray was applied in bands across the streams at 200-yard intervals. Applications were made before heavy foliage appeared in the thickly wooded areas, in order to insure maximum amounts of spray reaching the streams. Light to moderate black fly breeding was present in streams at the time of treatment. Thirty days later young turkeys were moved from the brooders and placed in range pens.

Numerous checking stations were established in each of the streams in the test plots, and the effectiveness of the treatments was determined by comparing counts of larvae attached to aquatic vegetation, dead leaves, and other debris before and after treatment. Counts were made 24 and 48 hours after treatment and weekly thereafter until the experiments were finished. Weekly counts were also made at untreated breeding sites several miles distant from each test plot.

Prior to treatment adult black fly populations were determined by hour-long observations of breeder turkeys on the pastures, and also of isolated turkeys staked out near black fly breeding areas. After treatment adult populations were checked in a similar manner on young poults. The incidence of *L. smithi* infections was determined by weekly microscopic examinations of blood smears from 25 or 50 birds in each range pen.

TESTS ON LARGE PLOT.—Preliminary surveys of the streams were made in January and again in February. Larvae of *Cnephia pecuarum* (Riley),[3] *Simulium congareenarum*, and members of the *S. venustum* complex[4] were collected in January, and larvae and pupae of these species in February. However, adult black flies were not apparent during these months. Early in March the larvae of *congareenarum* and the *venustum* complex were most abundant. The pupal stage was the predominant form of *pecuarum*. Adults of this species were collected in several locations by sweeping over emergence areas.

[1] Accepted for publication June 16, 1958.
[2] The Poultry Department of the Cypress Woods Corporation assisted in these studies.
[3] Alan Stone, of the Entomology Research Division, made the initial identification of the black flies reported herein.
[4] The *S. venustum* complex includes true *venustum* Say, *tuberosum* (Lundst.), and *verecundum* Stone & Jamback. Since these species were not collected from turkeys and apparently not concerned with disease transmission, identification beyond the complex was not attempted.

Larvae and pupae of *S. decorum* Walker were collected from the spillway of a dam near the poultry pastures. Of these species only *congareenarum* was known to feed on turkeys. A known vector of *L. smithi* in Jasper County, *slossonae*, was not found during any of the pretreatment surveys but appeared in untreated streams the first week in April.

Pretreatment counts of adults showed very low populations on the breeder turkeys. Only nine adults, all *congareenarum*, were collected during nine observation periods. Blood smears prepared from a representative number of these turkeys after the breeding season showed only 10% harboring gametocytes of *L. smithi*. As an additional measure to eliminate reservoir infection in the test plot, these breeders were sold and removed from the farm before the poults of the first hatch were placed on the pastures.

As shown in table 1, the larvicide applications gave almost complete control of black fly larvae for 2 weeks but only 72 and 68% control during the third and fourth weeks. By the fifth week water was flowing at only three of 10 checking stations, and these unfavorable breeding conditions were partly responsible for an indicated 96% control. During the next 8 weeks less than 2 inches of rain fell in the treated plot, and black fly breeding was practically nonexistent except for brief periods following showers which caused the streams to resume flowing. Only eight pupae, all *venustum* or closely related species, were found in the entire plot during this period.

Approximately 2000 6-weeks-old broad-breasted bronze turkey poults were placed on the pasture in one range pen the fourth week after treatment. Table 1 shows that blood smears prepared at the time and a series of smears 1 week later were both negative. After 2 weeks on the range 10% of the turkeys were infected and the incidence increased each week thereafter. Poults from other hatches were placed in range pens at weekly intervals, and the smears from each group showed a similar trend of infection.

That black flies were adequately controlled in the plot by the combination of the insecticide treatment and drouth is further supported by the results of the daily range observations. The turkeys were observed twice daily during their first 2 weeks on the range, and no black flies were seen feeding on them. When the first infections were noted, observations were increased to four times daily—at dawn, approximately 10 A.M., approximately 3 P.M., and at dusk. These studies also failed to reveal black flies. Sand flies (*Culicoides* spp.) were collected on three occasions at dawn, and mosquitoes (*Aedes sollicitans* (Walker) and *taeniorhynchus* (Wied.)) were especially abundant at dusk during the latter half of the study. On two occasions the dawn observations revealed large numbers of engorged *sollicitans* and *taeniorhynchus* resting in the turkey shelters. *A. infirmatus* D. & K. and *atlanticus* D. & K. were also collected occasionally from isolated turkeys in wooded areas. A few tabanids, *Diachlorus ferrugatus* (F.) and *Chrysops univittata* Macq., were also taken from isolated turkeys.

Table 1.—Control of black fly larvae with DDT and the incidence of *Leucocytozoon smithi* in turkeys in two test plots.

Weeks After Treatment	Large Plot		Small Plot	
	Per Cent Control	Per Cent of Turkeys Infected	Per Cent Control	Per Cent of Turkeys Infected
1	99	—	95	—
2	99	—	95	—
3	72	—	87	0
4	68	0[a]	88	0
5	96	0	92	0[a]
6	99	10	92	0
7	95	20	99	56
8	99	45	99	100
9	93	60	100	—
10	100	80	100	—
11	100	80	—	—

[a] On range this week.

The apparent absence of immature black flies in the streams and the failure to find adult black flies feeding on the turkeys suggest the possibility that other insects may also serve as vectors of *L. smithi*. A similar observation reported by Savage & Isa (1945) noted a severe outbreak of *Leucocytozoon* disease in Canada with the absence of black flies. In this outbreak, a competent entomologist reported innumerable mosquitoes and an abundance of flies, mostly of the genus *Stomoxys*.

TESTS ON SMALL PLOT.—Pretreatment stream surveys revealed moderate to heavy breeding of *congareenarum*, the *venustum* complex, and *pecuarum* in the streams, but adults were not collected. The plot was sprayed the second week in March. As shown in table 1, post-treatment examinations showed 95% control of larvae after the first and second weeks, and 87 and 88% after the third and fourth weeks. During the fifth and sixth weeks the streams were flowing at only two of the checking stations, but larvae and pupae of *congareenarum* and *slossonae* were present at both. The indicated control of larvae was approximately the same as that in the larger plot, but pupae were taken at every collection period through the eighth week, after which time the streams stopped flowing entirely.

Twenty-five poults 6 weeks of age were placed in this plot 3 weeks after treatment. Pasture facilities were unsatisfactory; so the young turkeys were kept in an enclosed shelter another 2 weeks until the fences were completed. Blood smears were prepared from the turkeys on the date of arrival and weekly thereafter. The smears were negative initially and also through the fourth week. Infections became apparent in the fifth week, and by the sixth week all the turkeys were infected. The turkeys were observed only during the weekly bleeding periods, but adult black flies were not seen at these times.

Since the larvicides failed to stop black fly breeding in this plot, the occurrence of *Leucocytozoon* disease was not surprising. Black flies were not seen on the turkeys during the brief bleeding periods, but it is possible that a few were active at dawn or dusk. In any event the presence of S. *congareenarum* and *slossonae* pupae in nearly every weekly collection supports this possibility and suggests that one or both species probably were responsible for at least some of the disease transmissions.

REFERENCES CITED

Johnson, E. P., G. W. Underhill, J. A. Cox, and W. L. Threlkeld. 1938. A blood protozoan of turkeys transmitted by *Simulium nigroparvum* (Twinn). Amer. Jour. Hyg. **27**: 649–65.

Jones, Calvin M., and Dale J. Richey. 1956. Biology of the black flies in Jasper County, South Carolina and some relationships to a *Leucocytozoon* disease of turkeys. Jour. Econ. Ent. **49**(1): 121–3.

Savage, A., and J. M. Isa. 1945. A case report: An outbreak of *Leucocytozoon* disease in turkeys. Cornell Vet. **35**(3): 270–2.

Skidmore, L. V. 1932. *Leucocytozoon smithi* infection in turkeys and its transmission by *Simulium occidentale* Towns. Zentbl. Bakt. **1-125**: 329–35.

Underhill, G. W. 1939. Two simuliids found feeding on turkeys in Virginia. Jour. Econ. Ent. **32**(6): 765–8.

U. S. Bureau of Animal Industry, Annual Report, 1953, p. 178.

Effects of Methyl Bromide Fumigation on the Viability of Barley, Corn, Grain Sorghum, Oats, and Wheat Seeds[1]

W. KEITH WHITNEY,[2] ORLO K. JANTZ,[3] and CARL S. BULGER, *Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agr. Mktg. Serv., U.S.D.A., Manhattan, Kans.*

ABSTRACT

Methyl bromide is a highly efficient fumigant, and is widely used for the fumigation of stored grain, mills, and warehouses. One of its undesirable qualities, at least for seed fumigation, is its phytotoxicity. Viability of seeds may be seriously impaired by excessive treatments with methyl bromide.

Tests were conducted to determine the effects of methyl bromide on the viability of barley, corn, grain sorghum, oats, and wheat seeds when fumigated at 80° F. with different combinations of dosage, exposure, and seed moisture content. Gas analyses were made at the end of each fumigation to verify the methyl bromide concentrations. Germination tests were conducted 24 hours, 30 days, and with wheat only, 6 months after fumigation to observe immediate and delayed effects. There was a pronounced decrease in viability in some cases with the increased period of storage after fumigation. In some experiments standardized seedling evaluations were made so that sublethal, injurious effects could be observed. Many of the fumigated seeds sprouted but did not develop normally.

A margin of tolerance usually exists between the dosages required for insect control and those which are lethal to high quality, dry seeds. This margin of tolerance is dependent upon the complex interaction of several variable factors, including (1) the fumigant dosage applied, (2) the seed moisture content, (3) the length of exposure, (4) the kind of seed, (5) the period and conditions of storage after fumigation, (6) the fumigation temperature, (7) the history of the seed (age, previous fumigations, etc.), (8) the ratio of commodity to total space in the fumatorium (the sorption capacity), and (9) leakage factors in the fumatorium. Some of these factors were explored here, some have been reported by other workers.

In general, the results of the study show that little or no injury occurred when the following combination of conditions existed: (1) the seed moisture was less than 12%, (2) the dosage was less than 2 lb./1000 cu. ft., (3) the exposure period was less than 24 hours, and (4) the temperature was 80° F. High temperature, moisture, dosage, and long exposure all contribute to seed injury from fumigation. When combinations of fumigation conditions occur in which one (or more) of these variables is of a higher order than named above, moderate to extensive germination damage may be expected.

The over-all relative order of tolerance of the five species tested was oats > barley > grain sorghum > corn > wheat.

It is commonly recognized that fumigation of seeds with methyl bromide may cause injury to their germination and growing powers. In view of the fact that methyl bromide is a highly effective fumigant, it is desirable to know how much of this gas different seeds can tolerate under different conditions without damage to viability. Several varied and scattered bits of this type of information have accumulated; however, to the knowledge of the authors, no co-ordinated extensive tests have been previously conducted. At the present time tests similar to those described in this paper are underway by Strong and Lindgren (unpublished data) at the University of California, Citrus Experiment Station, Riverside, Calif., and by King and Garner, Texas A. & M. College, College Station, Tex. Further tests with methyl bromide and other fumigants are in progress at the Manhattan, Kans., Stored-Product Insects Laboratory, where the tests reported herein were conducted.

EXPERIMENTAL PROCEDURE AND MATERIALS.—*Seeds.*—These kinds of seeds were used: Barley (*Hordeum vulgare* L.), Beecher variety, six-row, smooth spring. Corn (*Zea mays* L.), DeKalb, yellow, hybrid. Grain sorghum (*Sorghum vulgare* Pers.), Midland variety, yellow, combine type. Oats (*Avena sativa* L.), Nemaha variety, white, spring. Wheat (*Triticum aestivum* L.), Pawnee variety, Hard Red Winter (Class IV).

Moisture Contents of Seeds.—Before fumigation of the seeds, the moisture content of various lots of each kind of seed was adjusted to 10, 11, 12, 13, and 14%. The moisture content was increased by introducing a calculated amount of distilled water by pipette to seeds held at room temperature in a 2-quart glass jar. The jar was then sealed and rolled a few minutes each day for 1 week to mix the seeds and water. Moisture was decreased,

[1] Portion of a thesis presented in partial fulfillment of the requirements for the degree Master of Science in Entomology at Kansas State College. Accepted for publication June 17, 1958.

[2] Present address: Department of Entomology, Kansas State College, Manhattan, Kansas. The valuable counsel of Mr. H. H. Walkden, Station Leader, Stored-Product Insects Laboratory, Manhattan, Kans., and of Prof. D. A. Wilbur, major instructor for the senior author, is acknowledged with sincere appreciation.

[3] Now on military leave in the U. S. Air Force.

when necessary, by drying seeds in a forced-air oven at 90° F. Moisture measurements were made by use of a Steinlite[4] Moisture Tester. The manufacturer of this tester claims it has an accuracy of ±0.25%. Other investigators (Hlynka & Anderson 1949) found the error of estimate to be ±0.4%. Since no method was available to control the atmospheric humidity in the laboratory, the moisture contents of the seeds were not maintained at their original levels after fumigation.

Fumigation of Seeds.—Five-gallon glass bottles were used as the fumatoria (fig. 1). Small bags made of open-mesh rayon curtain scrim were used to contain the grain. Of the small grains, 100 grams of seeds were used in each bag; 50 grams of corn were used in each bag. These seed-filled bags were suspended in the center of the fumatoria. The number of bags in each bottle depended upon the number of moisture levels being tested. Seeds of each moisture level were placed in a separate bag, but one bag of each was placed in a bottle. The ratio of seeds to air space thus approximated that which would normally be found in a large warehouse partially filled with bags or small bins of seeds.

Methyl bromide was applied at the rates of 2, 4, 6, and 8 pounds per 1000 cu. ft. in all tests and at 0.6, 1, 3, and 5 pounds per 1000 cu. ft. in a few additional tests with wheat. The fumigant was measured at 0° F. in the liquid state by a cold pipette and was introduced into the fumigation bottle by releasing a partial (about 15 inches of mercury) vacuum created in the bottle just prior to fumigation. The air rushing through the stopcock and into the bottle greatly aided in vaporizing the liquid methyl bromide and dispersing the gas in the bottle. The fumigations were conducted at atmospheric pressure, 80° and for exposures of 4, 8, 12, and 24 hours.

FIG. 1.—Seeds held in small bags (A) were fumigated in 5-gallon glass bottles as shown. The glass stopcock (B) and neoprene stopper (C) were used to admit the fumigant and to close the bottle.

Gas Analyses.—Methyl bromide concentrations were determined immediately before removal of the seeds from each fumatorium. This was done by use of a Gow-Mac thermal conductivity gas analyzer. This method of gas analyses is fully discussed by Phillips & Bulger (1953), Monro *et al.* (1953), and Phillips (1957).

Aeration of Seeds.—After removal from the fumatoria, the seeds were aerated for 24 hours by exposing them to the laboratory atmosphere in the open-mesh seed bags used in the fumigation. The portion of each sample not used in the first germination test was held in a 4-ounce glass jar for later tests. Each jar was covered with a screen lid to permit further aeration of the seeds and also to prevent entry of insects.

Germination Tests.—Germination tests were conducted at three different time intervals after fumigation so that immediate and delayed effects could be observed. These tests were started 1 day, 30 days, and with wheat only, 6 months after fumigation. Only whole, undamaged seeds were used for the germination tests.

Two Mangelsdorf germinators were used. Except for a few preliminary tests, the general germination technique and procedures followed those recommended by the U. S. Department of Agriculture (1952). Standardized seedling interpretations were made for one complete test series with wheat in which the seeds were evaluated as to "normal," "abnormal," or "dead." In all other tests, seeds which showed any sign of embryonic development, however slight, were counted as "germinated." According to the standardized seedling interpretations, only normal seedlings are considered germinated. Sprout counts were made after 5 and 10 days' incubation for the standardized seedling series. Normal seedlings found in the 5-day counts were removed from the blotters, while others were left in place for further incubation. A 7-day count was made for each of the samples where seedlings were not evaluated.

Mold Inhibitor.—Mold growth was a problem, especially in samples severely injured by the fumigant. After it was determined that no apparent difference was caused in seed viability by its use, a suspension of Captan (N-trichloromethyl thio tetrahydrophthalimide) in water was applied to the germination blotters before placement of the seeds.

Replicates.—Each fumigation was duplicated in nearly every test series. In some instances several replicate fumigation series were conducted. The numbers of seeds used in the germination tests ranged from 200 to 4100 for each dosage, moisture level, exposure, and kind of seed. These numbers are indicated in the tabular data.

Tabulation and Evaluation of Data.[5]—Results of in-

[4] The use of trade names in this paper is for identification purposes only and does not constitute an endorsement of the product by the United States Department of Agriculture.

[5] The authors are grateful to H. D. Nelson, Assistant Station Leader, Stored-Product Insects Laboratory, Manhattan, Kans., who performed the statistical analyses under the counsel of Dr. H. C. Fryer, in charge of the Statistics Laboratory, Kansas State College, Manhattan.

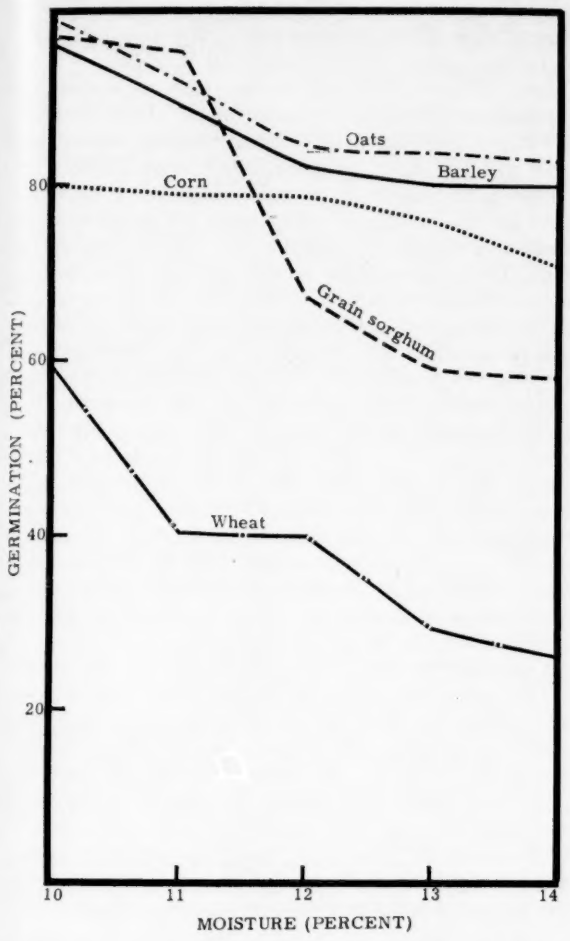


FIG. 2.—An example of the effects of seed moisture content during fumigation on germination (30-day test, 24-hour exposure, methyl bromide 2 lb./1000 cu. ft.).

dividual samples were calculated as percentages and analyzed by the analysis of variance. Least significant differences were computed at the 5% level (L.S.D.*). In some instances, as when a sample was lost or insufficient seeds were available for a complete series, results for the missing individual samples were estimated by an unbiased method. This was done so that each series would have equal numbers of samples, thus simplifying the work involved in the analysis of variance. Wheat data, except the selected data, are expressed in terms of corrected differences between the treated samples and the checks. This correction was made by use of Abbott's formula to account for abnormal and dead seeds in the check samples.

RESULTS.—*General.*—The data presented in this report reinforce, delineate, and extend the existing rather empirical knowledge of the facts relating to the effects of methyl bromide fumigation on seed viability. Injury to seeds was found to be directly related to increases in (1) fumigant concentration, (2) length of exposure, (3) seed moisture content, and (4) length of storage period after fumigation. From other work by Cotton and Frankenfeld (unpublished data), it was demonstrated that the degree of injury increased in close association with increasing fumigation temperatures.

The rates of increasing injury are not uniformly correlated with increases in the variable factors named above, but certain critical thresholds exist. These thresholds are so complexly interdependent that a minor change in one variable may cause the whole system to shift considerably.

Although seedling evaluations were not made for all of the kinds of seeds tested, it was apparent that many of the sprouts from fumigated seeds were slow in developing and were often malformed. This was especially notable in the higher dosages and longer exposures. These observations were confirmed by the seedling evaluations with wheat.

Barley.—Figures 2, 3, and 4 illustrate the fact that barley was one of the more resistant kinds of seed. A summary and least significant differences are presented in table 1 and shows that there was a significant interaction among all the variables involved; namely, dosage ×exposure×moisture×the time interval between fumigation and germination test (germination test number).

A few examples are cited from table 1 in the following

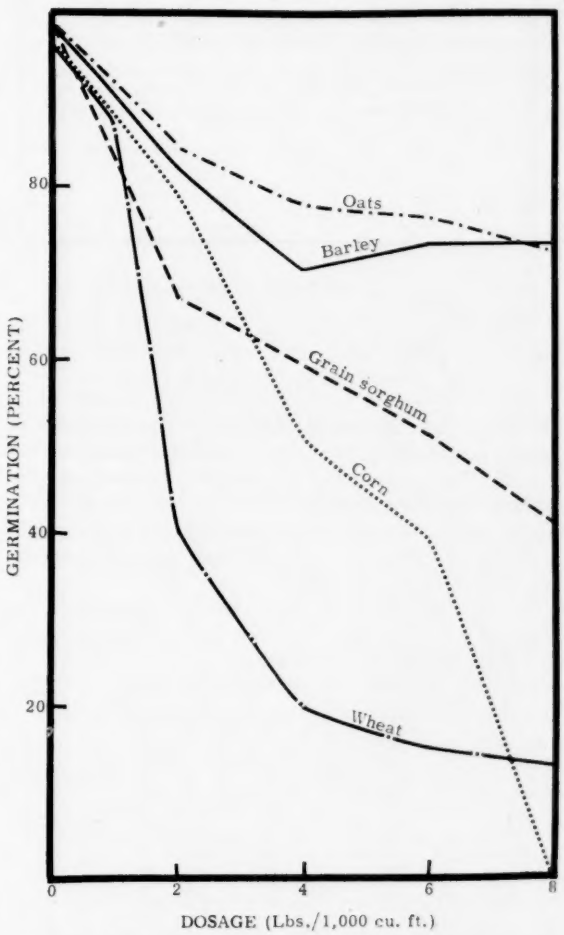


FIG. 3.—An example of the effects of fumigant dosage on seed germination (30-day test, 12% moisture, 24 hours exposure to methyl bromide).

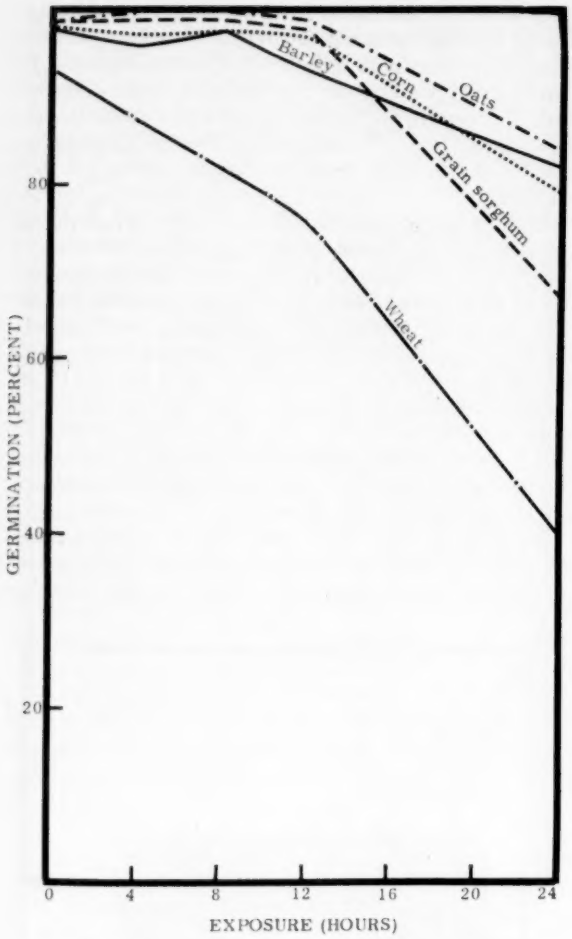


FIG. 4.—An example of the effects of length of fumigant exposure on seed germination (30-day test, 12% moisture, methyl bromide 2 lb./1000 cu. ft.).

tabulation. This tabulation shows the combinations of variables in which significant germination reductions first occurred. The comparisons are between treated samples and their respective checks (L.S.D.* = 6.3). Except for a few instances, all combinations of conditions in which any one of the variables was greater than listed below also resulted in significant injury.

Dosage (Lb./1000 cu. ft.)	*Exposure (Hours)*	*Moisture (Per Cent)*	*Germination Test No.*	
			First	*Second*
2	12	13	x	x
2	24	11	x	x
4	8	11		x
4	12	10	x	x
6	4	13		x
6	4	14	x	x
6	8	10		x
8	4	11	x	x
8	8	10	x	x

As indicated in column 2 of table 1, gas analyses at the end of each fumigation showed that the fumigant concentrations were quite close to the amounts applied.

The results of the barley tests compare favorably with those found by other workers (Fisk & Shepherd 1938, Mackie 1938, Mayer & Gammon unpublished data, California State Senate 1955, Lindgren *et al.* 1955).

Corn.—Figures 2, 3, and 4 show some representative germination values for corn after methyl bromide fumigation, and also the relative susceptibility of corn as compared with the other species tested. As shown in figure 2, increasing the moisture content above 12% caused a slight increase in susceptibility when the seeds were exposed to a dosage of 2 pounds per 1000 cu. ft., for 24 hours. The 12% moisture level is apparently the second moisture threshold with corn, since the treated seeds having only 10% moisture germinated 80% while the checks germinated about 99%. Figure 3 indicates that corn was very sensitive to increasing dosages. The dosage threshold for 12% moisture and 24 hours' exposure lies some place below 2 pounds per 1000 cu. ft. The exact point is not known because no dosages less than 2 pounds were tested in corn. Figure 4 expresses the relationship between increasing lengths of exposure and damage to viability when the dosage was 2 pounds per 1000 cu. ft. and the moisture was 12%. Under these conditions the treatments were tolerated until exposures greater than 12 hours were used. The reaction of corn to increasing exposures was similar to that of oats, barley, and grain sorghum.

A summary and statistical statement are given in table 2. The interaction among all the variables involved was not significant. Interactions of moisture×dosage×exposure and of germination test number×dosage×exposure were significant. The data were arranged according to the combinations just listed, and L.S.D.* values were computed. The following tabulation cites examples of combinations of variables in which significant reductions in germination first occurred. The comparisons are between treated samples and their respective checks.

Dosage (Lb./1000 cu. ft.)	*Exposure (Hours)*	*Moisture (Per Cent)*	*Germination Test No.*		*L.S.D.**
			First	*Second*	
(Moisture×Dosage×Exposure)					
2	24	10	(Combined)		8.1
4	8	10	(Combined)		5.9
6	4	13	(Combined)		8.1
6	8	10	(Combined)		8.1
8	4	10	(Combined)		8.1
(Germination test number×Dosage×Exposure)					
2	24	(All combined)	x	x	5.3
4	8	(All combined)	x	x	3.7
6	4	(All combined)		x	5.3
6	8	(All combined)	x	x	5.3
8	4	(All combined)	x	x	5.3

Combinations of variables in which any one factor was increased above those listed resulted in increased injury. Some of the more severe treatments reduced germinations nearly to zero.

Thermal conductivity gas readings at the end of each fumigation indicated that even though the samples were not large the seeds had sorbed a small amount of the methyl bromide during the 24-hour exposure periods.

Table 1.—Beecher barley: effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure (Hours)	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
		10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
2 lb./1000 cu. ft.											
Untreated check	—	99.7	98.0	99.7	99.3	99.3	97.7	99.0	98.7	99.0	99.0
4	27	99.4	97.8	99.4	99.2	99.2	96.4	99.0	99.2	98.4	97.0
8	28	98.6	99.2	97.4	97.6	97.2	98.2	97.2	95.2	98.2	97.8
12	29	97.8	98.2	98.2	97.8	93.4	93.4	90.0	91.2	93.0	87.2
24	30	94.8	95.6	91.4	88.8	82.4	81.6	81.2	79.8	79.2	79.8
4 lb./1000 cu. ft.											
Untreated check	—	98.3	97.0	100	99.3	97.3	96.7	93.3	87.7	90.7	88.3
4	65	99.0	97.4	98.4	96.2	97.2	91.4	96.0	87.4	97.0	90.4
8	62	98.8	93.4	95.8	84.2	93.4	77.4	87.8	74.4	92.0	74.4
12	62	87.0	84.8	85.4	77.8	81.8	76.2	77.6	75.8	75.0	73.2
24	62	72.8	72.8	74.6	74.6	75.0	69.8	75.2	77.8	71.0	72.6
6 lb./1000 cu. ft.											
Untreated check	—	97.7	98.3	99.7	97.0	99.3	98.3	98.3	95.6	99.3	96.7
4	99	98.4	95.4	98.0	93.2	95.8	94.6	92.6	85.0	91.8	84.2
8	102	94.4	89.4	81.8	79.4	79.8	80.0	78.2	73.4	78.0	75.2
12	99	86.8	86.4	79.8	81.2	76.6	79.6	80.0	73.0	71.2	72.0
24	101	79.6	78.8	74.8	77.2	70.8	72.8	70.0	70.2	71.2	70.8
8 lb./1000 cu. ft.											
Untreated check	—	99.3	97.0	98.2	97.0	98.7	95.3	96.0	95.0	98.3	94.7
4	132	94.6	92.4	84.0	84.8	81.2	84.4	87.2	76.2	75.4	72.0
8	128	85.6	80.6	72.8	71.6	74.8	79.8	76.0	77.4	65.8	57.2
12	128	74.8	75.2	73.4	76.8	76.6	72.6	70.6	76.8	52.6	67.6
24	127	74.2	74.2	71.2	73.2	71.6	73.4	68.8	73.2	45.6	49.2

[a] The fumigation temperature was 80° F. Five hundred seeds tested under each set of conditions except that 300 seeds were tested in the untreated checks.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
Least significant mean differences at the 5% level (L.S.D.*).
Comparing samples having 300 seeds, L.S.D.* = 7.0.
Comparing samples having 300 seeds and samples having 500 seeds, L.S.D.* = 6.3.
Comparing samples having 500 seeds, L.S.D.* = 5.1.

Readings at other times were well within the usual limits of variation (table 2, column 2).

A series of tests was conducted by Frankenfeld in 1939 (unpublished data), the results of which indicate that corn had a higher degree of tolerance than shown in this paper. The technique used by Frankenfeld differed from that used in these tests in that the ratio of corn to free air space was much higher in the former, simulating that which is found in bulk storage. As mentioned earlier, in the tests reported here the ratio of seeds to free air space approximated that which would be found in a large warehouse partially filled with bags or small bins of seeds. It is believed that this higher corn-to-air space ratio in Frankenfeld's tests caused a greater amount of the gas to be held by sorption, thus accounting for the higher tolerance indicated. Other factors, such as seed variety, may also effect these differences.

Grain Sorghum.—In general, grain sorghum appeared to be more tolerant than wheat and less tolerant than barley and oats. Figure 2 shows that sensitivity to 2 pounds/1000 cu. ft. for 24 hours was greatly increased when seed moisture was increased above 11%. When compared with corn under these conditions, the tolerance of grain sorghum was considerably above corn at moistures of 10% and 11% but dropped below corn at moisture levels of 12, 13, and 14%. This indicates that at a 2-pound dosage and 24 hours' exposure grain sorghum was more sensitive than corn to moisture changes.

Figure 3 shows that grain sorghum (12% moisture and 24 hours' exposure) was more sensitive than barley or oats to increasing fumigant dosage, but less sensitive than wheat or corn.

Increasing exposures (fig. 4) were tolerated by grain sorghum (12% moisture and 2-pound dosage) through 12 hours' exposure, but serious damage resulted from 24 hours' exposure.

The breaking points (thresholds) varied in relation to the combinations of all factors involved and may be found for each combination by studying table 3. Analysis of variance revealed a significant interaction among the four factors involved; namely, moisture×dosage×exposure×germination test (delayed effect). The L.S.D.* was found to be 5.5 when comparing treated with untreated samples (table 3). On the basis of this criterion, the following tabulation cites examples of combinations of variables listed in table 3 in which significant germination reductions first occurred:

Dosage (Lb./1000 cu. ft.)	*Exposure (Hours)*	*Moisture (Per Cent)*	*Germination Test No.*	
			First	*Second*
2	24	12	x	x
4	8	12		x
4	12	10	x	x
6	8	12	x	x
6	12	10	x	
8	4	11	x	x
8	8	10	x	x

Table 2.—Yellow hybrid corn: effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure (Hours)	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Contents (1st and 2nd Tests Combined) 10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] in Indicated Germination Test[c] (All Moisture Levels Combined) Test 1	Test 2
2 lb./1000 cu. ft.										
Untreated check	—	400	99.3	100	99.0	99.3	99.5	1000	99.7	99.1
4	32	400	100	98.8	97.8	98.0	99.3	1000	99.1	98.4
8	32	400	99.0	97.8	98.0	96.8	97.3	1000	99.1	96.4
12	33	400	98.8	98.0	95.3	96.8	91.8	1000	97.2	95.0
24	27	400	79.5	81.3	78.5	74.8	72.3	1000	77.5	77.0
4 lb./1000 cu. ft.										
Untreated check	—	800	98.1	94.0	98.1	98.9	99.6	2000	98.0	97.5
4	64	800	98.6	97.5	96.6	96.4	96.8	2000	97.6	96.8
8	64	800	89.4	84.0	78.8	81.0	70.6	2000	78.7	82.9
12	61	800	76.5	74.4	70.9	69.5	56.9	2000	69.3	70.0
24	55	800	73.3	64.8	47.5	28.6	11.5	2000	45.2	45.0
6 lb./1000 cu. ft.										
Untreated check	—	400	99.3	100	99.5	99.5	100	1000	99.8	99.5
4	97	400	97.5	96.3	92.3	89.8	89.5	1000	94.9	91.2
8	97	400	83.0	76.0	79.5	67.3	62.8	1000	77.5	69.9
12	94	400	75.8	76.3	75.3	37.8	49.0	1000	69.3	56.3
24	92	400	71.0	68.3	71.8	2.3	7.5	1000	46.2	34.1
8 lb./1000 cu. ft.										
Untreated check	—	400	99.3	99.5	99.5	99.3	100	1000	99.8	99.2
4	126	400	90.8	86.8	81.3	80.8	84.3	1000	87.6	81.9
8	125	400	80.3	81.8	72.8	66.8	67.3	1000	77.4	73.1
12	126	400	76.5	73.3	44.3	41.8	60.3	1000	72.5	45.9
24	124	400	60.5	50.5	0.8	2.0	16.3	1000	31.9	20.1

[a] The fumigation temperature was 80° F.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
Least significant mean differences at the 5% level (L.S.D.*) when considering these interactions:
Moisture×Dosage×Exposure:
Comparing samples having 400 seeds, L.S.D.*=8.1.
Comparing samples having 400 seeds with samples having 800 seeds, L.S.D.*=7.2.
Comparing samples having 800 seeds, L.S.D.*=5.9.
Test Number×Dosage×Exposure:
Comparing samples having 1000 seeds, L.S.D.*=5.3.
Comparing samples having 1000 seeds with samples having 2000 seeds, L.S.D.*=4.6.
Comparing samples having 2000 seeds, L.S.D.*=3.7.

Combinations of variables in which any one factor was increased above those listed resulted in increased injury. Results of gas analyses at the end of each fumigation indicated that the methyl bromide concentrations were approximately equal to the amount applied.

Experiments reported by Lindgren *et al.* (1955) indicate similar results as reported above.

Oats.—Oats were more tolerant to methyl bromide than any other seed tested, as shown in figures 2, 3, and 4. Increasing the moisture, dosage, and exposure caused moderate increases in damage, but not to the extent observed with the other seeds tested.

Statistical analysis of the data disclosed that significant interactions occurred only at the 2-way level; namely, dosage×exposure, moisture×exposure, and dosage×germination test number. A summary of the individual tests, arranged according to the combinations having significant interactions, is given in table 4. By using the appropriate least significant difference values which are listed at the bottom of table 4, comparisons can be made in the table to show which treatments caused injury and also whether differences existed between first and second germination tests.

The following tabulation cites those combinations of variables in which significant injury first occurred:

Dosage (Lb./1000 cu. ft.)	*Exposure (Hours)*	*Moisture (Per Cent)*	*L.S.D.**
	(Dosage×Exposure)		
2	24	All combined	5.4
4	8	All combined	5.4
6	4	All combined	5.4
	(Exposure×Moisture)		
All combined	12	10	6.0
All combined	8	11	6.0
All combined	4	13	6.0

Each combination in which either factor was increased resulted in increased injury; those with smaller factors were not damaged. Relationships of germinations in the first tests (24 hours' aeration) and the second tests (30 days' aeration) were somewhat erratic; however, two definite trends are shown. The 4-pound dosage (all moisture levels and exposures combined) caused a significantly greater reduction in the first test than was found in the second test. The reverse situation was true for samples treated with the 8-pound dosage.

Table 5 lists average germinations for each combination of moisture×exposure×dosage×germination test number. Although the interaction at this level was not significant, it is obvious that the following are the approximate upper limits of tolerance:

Dosage (*Lb./1000 cu. ft.*)	*Exposure* (*Hours*)	*Moisture* (*Per Cent*)
2	24	11
4	8	11
4	12	10
6	4	12
8	4	11

In fact, some of the examples just cited sustained moderate amounts of injury. In each case, an increase in one of the variables resulted in damage.

Gas analyses at the end of each of the exposures corroborate the theoretical dosages, since they lie within the usual limits of experimental error.

The results of the tests with oats agree with the preliminary findings of Fisk & Shepherd (1938) and those in the khapra beetle research (California State Senate 1955).

Wheat.—General.—Wheat was definitely the most susceptible species tested. Several hundred seeds were used in each of the test series with wheat, partly because of its erratic response and also because wheat viability is of great economic importance. Flour millers do not want dead wheat. There is experimental evidence that shows germ damage from fumigation is directly associated with inferior bread quality when made from flour of damaged wheat (Cotton *et al.* 1946).

Figure 2 illustrates the fact that the sensitivity does not always change in direct and uniform proportions to changes in moisture content. It appears that certain critical moisture thresholds exist here also which are, obviously, dependent upon the combination of the other variables involved. Similar thresholds undoubtedly occur for the other variables, too, but in the combinations used for the data in figures 3 and 4, they are not readily apparent. Figures 3 and 4 indicate that injury to wheat is more uniformly related to dosage and exposure than to moisture, under the conditions of these tests.

Interactions of Variable Factors.—Graphic representations of the germination results from seeds treated with various combinations of dosage, moisture, and exposure aid in giving a clearer understanding of these interactions. Figures 5, 6, and 7 present a few such combinations of summarized data.

The relationship of dosage×moisture×germination is illustrated in figure 5. During a 4-hour exposure, significant, but not drastic, reductions in viability resulted from treatments of 6-pound dosage×14% moisture and of 8 pounds×10% and 11% moisture levels. Severe injury was associated with the 8-pound treatment of seeds having 12, 13, and 14% moisture levels.

Data showing the association of dosage×exposure ×germination are portrayed in figure 6. Germination of

Table 3.—Midland grain sorghum: effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure (Hours)	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
		10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
2 lb./1000 cu. ft.											
Untreated check	—	97.3	99.7	97.7	98.7	99.3	99.0	98.7	99.0	98.0	98.7
4	27	98.2	98.6	96.8	98.2	98.2	98.6	98.2	98.8	98.0	97.8
8	28	98.4	99.4	98.6	98.6	97.6	99.6	99.0	99.0	95.4	97.8
12	29	99.4	98.4	97.6	98.8	97.4	97.6	96.2	98.0	97.8	95.6
24	30	97.4	96.6	94.6	96.2	70.0	66.8	63.2	58.8	57.6	58.2
4 lb./1000 cu. ft.											
Untreated check	—	98.7	98.7	98.0	97.7	97.7	97.7	98.3	98.3	97.7	99.3
4	65	99.0	99.0	99.0	99.0	98.4	97.8	99.2	96.8	98.6	98.8
8	62	98.8	97.4	94.6	98.0	92.4	91.2	62.4	75.6	71.6	75.4
12	62	90.0	91.8	65.8	85.6	57.8	64.4	40.4	54.0	57.6	50.4
24	62	56.2	58.0	55.6	55.6	57.4	59.0	31.0	22.0	2.2	2.6
6 lb./1000 cu. ft.											
Untreated check	—	99.0	99.7	99.3	98.3	99.7	99.3	99.0	98.7	98.0	99.7
4	99	97.4	98.4	98.8	98.6	97.2	98.4	97.2	95.8	97.4	97.6
8	102	98.2	97.6	96.4	96.4	70.2	80.6	54.4	60.6	45.8	48.8
12	99	93.0	97.4	61.8	80.0	56.8	61.4	50.2	47.0	16.4	16.4
24	101	56.0	56.2	52.4	56.0	51.6	51.4	0	1.6	0	0
8 lb./1000 cu. ft.											
Untreated check	—	99.0	99.0	98.7	100	98.3	100	98.0	99.7	99.7	98.7
4	132	95.2	97.4	90.0	94.8	90.4	94.0	87.2	85.8	67.0	79.2
8	128	79.8	68.4	58.4	60.2	59.4	54.8	50.0	57.6	1.2	0.2
12	128	57.8	62.4	58.4	61.6	50.8	57.2	20.2	18.6	0	0
24	127	54.2	58.2	47.2	58.6	44.8	40.8	0.2	0	0	0

[a] The fumigation temperature was 80° F. Five hundred seeds tested under each set of conditions except that 300 seeds were tested in the untreated checks.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
Least significant mean differences at the 5% level (L.S.D.*):
Comparing samples having 300 seeds, L.S.D.* = 6.1.
Comparing samples having 300 seeds with samples having 500 seeds, L.S.D.* = 5.5.
Comparing samples having 500 seeds, L.S.D.* = 4.7.

Table 4.—Nemaha oats: effects of methyl bromide fumigation[a] on seed germination. Summary of individual tests.

Length of Exposure (Hours) and Germination Test[b]	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination After Indicated Dosage (Lb./1000 Cu. Ft.)				Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination at Indicated Moisture Contents				
		2 Lbs.	4 Lbs.	6 Lbs.	8 Lbs.		10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent
Untreated checks	3000	99.7[c]	99.0	99.2	99.1	2400	99.1[d]	99.4	99.6	99.1	99.0
4	5000	99.6	98.7	89.4	88.1	4000	99.2	95.6	93.9	92.1	91.2
8	5000	99.4	89.4	82.1	78.7	4000	95.2	87.3	85.5	85.5	83.4
12	5000	98.4	83.9	78.2	75.8	4000	89.5	83.7	84.5	83.1	79.5
24	5000	86.7	72.5	71.7	64.6	4000	77.9	77.2	77.2	74.8	60.9
First test	11500	95.9[e]	86.4	83.6	81.1						
Second test	11500	97.1	89.2	82.8	78.3						

[a] The fumigation temperature was 80° F.
[b] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
[c] All moistures and first and second germination tests combined for each value given in this section.
[d] All dosages and first and second germination tests combined for each value given in this section.
[e] All moistures and exposures combined for values given in this section.
Least significant mean differences at the 5% level (L.S.D.*) when considering these interactions:
Dosage×Exposure:
Comparing samples having 3000 seeds, L.S.D.* = 6.0.
Comparing samples having 3000 seeds with samples having 5000 seeds, L.S.D.* = 5.4.
Comparing samples having 5000 seeds, L.S.D.* = 4.7.
Moisture×Exposure:
Comparing samples having 2400 seeds, L.S.D.* = 6.6.
Comparing samples having 2400 seeds with samples having 4000 seeds, L.S.D.* = 6.0.
Comparing samples having 4000 seeds, L.S.D.* = 5.2.
Dosage×Test:
L.S.D.* = 2.2.

Table 5.—Nemaha oats: effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure (Hours)	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
		10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
2 lb./1000 cu. ft.											
Untreated check	—	100	100	99.7	100	99.7	99.3	99.7	100	99.3	99.7
4	35	99.2	100	99.8	100	99.6	100	99.8	99.2	98.6	99.8
8	34	100	99.8	99.6	99.4	99.0	100	99.4	98.0	99.4	99.4
12	34	100	100	98.4	99.8	98.4	99.2	96.2	98.0	95.4	98.6
24	34	99.8	99.6	85.6	91.6	81.4	84.4	79.4	84.4	78.8	83.4
4 lb./1000 cu. ft.											
Untreated check	—	97.7	99.0	99.3	98.3	98.7	100	99.3	99.7	99.3	99.0
4	67	99.2	99.4	98.8	99.4	98.4	98.8	97.6	98.6	97.8	98.6
8	68	98.8	99.2	89.8	97.0	83.6	88.8	83.8	85.2	84.8	82.8
12	66	97.8	98.2	75.2	83.8	83.8	83.2	76.8	82.6	77.2	80.4
24	67	72.6	73.6	74.0	74.4	75.0	77.4	65.4	80.0	60.8	71.4
6 lbs./1000 cu. ft.											
Untreated check	—	99.0	99.3	99.7	99.7	99.3	100	96.0	100	99.3	99.3
4	98	99.0	99.0	93.2	89.8	94.6	88.2	89.2	86.0	85.0	88.0
8	98	86.4	91.4	84.2	84.4	82.6	76.2	80.6	82.2	76.6	76.2
12	96	76.2	79.0	78.6	82.2	80.2	78.0	85.0	76.8	73.2	72.8
24	96	72.4	75.4	75.2	74.0	78.8	75.8	76.8	72.8	59.4	56.4
8 lb./1000 cu. ft.											
Untreated check	—	99.0	99.0	99.7	99.0	100	100	99.0	99.0	99.3	96.7
4	134	99.4	98.8	95.2	88.2	89.8	81.8	87.6	78.8	80.4	81.4
8	132	96.4	89.8	69.6	74.6	78.8	75.0	78.0	76.6	74.4	73.4
12	132	85.2	80.0	75.6	76.2	79.2	73.8	79.6	70.0	73.4	65.2
24	125	73.8	69.2	70.0	72.6	72.0	72.4	70.4	68.8	39.6	37.6

[a] The fumigation temperature was 80° F. Five hundred seeds tested under each set of conditions except that 300 seeds were tested in the untreated checks.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
There are no significant interactions among the above data. Least significant differences are computed for those interactions which are significant and are presented in table 4.

wheat having 12% moisture was significantly, but not drastically, reduced by treatments of 2 pounds×8 hours and of 2 pounds×12 hours. The only samples surviving without injury were those in treatments of 2 pounds, 4 pounds, and 6 pounds×4 hours. All treatments other than those listed above caused great amounts of injury.

Interactions of moisture×exposure×germination are shown in figure 7. When fumigated with 2 pounds of methyl bromide per 1000 cu. ft., slight to moderate damage was done to samples in treatments of 4 hours×14% moisture, 8 hours×12, 13, and 14% moisture, and 12 hours×all moisture levels. Extensive reductions in germination resulted at all moisture levels×24 hours' exposure.

Summary of All Tests with Wheat.—The results of all tests conducted with wheat are summarized in table 6. All these data were not included in the statistical analyses because of the irregular and widely varying numbers of seeds used in each different set of conditions. Representative data were selected for statistical evaluation. The results of these selected data are summarized in table 7 and those of the seedling evaluations in table 8, both of which will be discussed subsequently.

A study of the corrected differences in germination between check and treated samples (table 6) shows that a

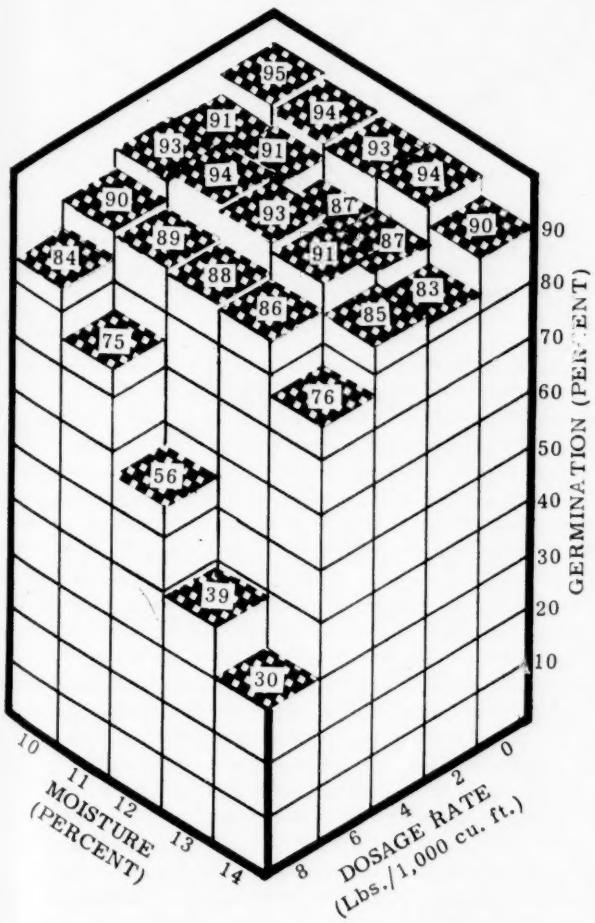


FIG. 5.—Data illustrating the interactions of seed moisture content and fumigant dosage on germination of Pawnee wheat 30 days after fumigation with methyl bromide for 4 hours at 80° F.

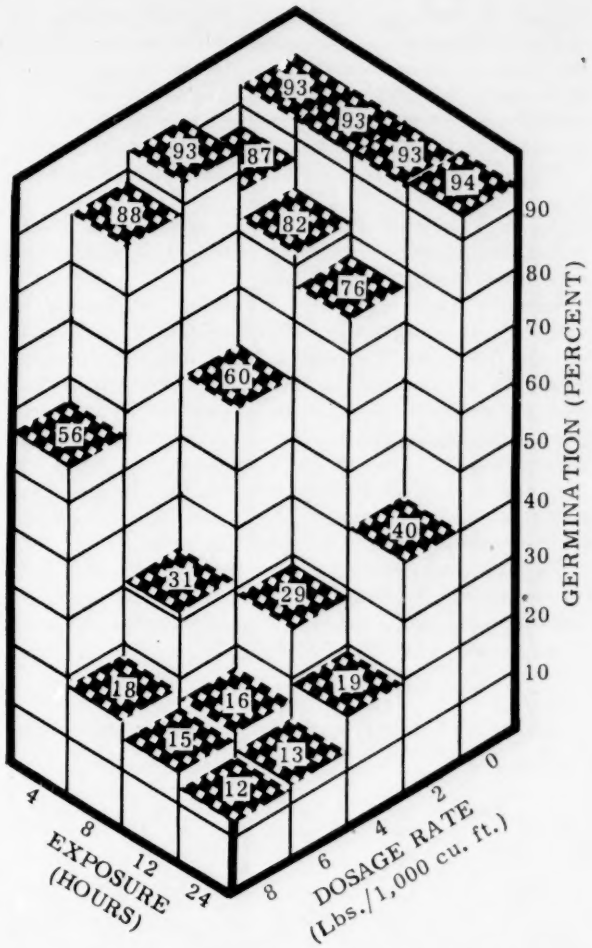


FIG. 6.—Data illustrating the interactions of length of fumigant exposure and dosage on germination of Pawnee wheat 30 days after fumigation with methyl bromide at 80° F. (moisture content of wheat 12%).

dosage of 0.6 pound per 1000 cu. ft. was tolerated by all samples, including those having 14% moisture which were exposed for 24 hours.

The 1-pound dosage caused slight injury in a few samples with the maximum reduction being 11%.

Two-pound dosages caused injury in proportion to the moisture content and the length of exposure. For example, seeds having 14% moisture were injured as follows: (third germination test, 6 months after treatment) 4 hours' exposure, 9% dead; 8 hours' exposure, 15% dead; 12 hours' exposure, 34% dead; and 24 hours' exposure, 70% dead. Lesser amounts of damage resulted when the seed moisture content was lower; however, slight to extensive injury occurred in nearly every sample.

The treatment with a 3-pound dosage×24-hour exposure was tolerated by the samples having 10% moisture, but considerable damage resulted in samples having 12% and 14% moisture.

Four-pound dosages were tolerated for 4 hours' exposure by all except 12% (or more) moisture samples, which sustained slight to moderate injury. Eight-hour exposures resulted in viability reductions ranging from

7% kill in the samples with 10% moisture to 55% kill in those samples with 14% moisture. The ranges of damage resulting from 12- and 24-hour exposures to dosages of 4 pounds per 1000 cu. ft. were 25% to 83% and 64% to 87%, respectively. The degree of injury again was dependent upon the moisture content of the seeds.

Five-pound dosages caused from 12 to 92% mortality of seed embryos.

Six- and 8-pound applications are not safe for wheat unless the moisture content is less than 10% and/or the exposure is shorter than 4 hours.

Selected Data.—Table 7, showing representative germination averages and L.S.D.* values for wheat, was constructed to aid in a more detailed study of the data. The data were selected in units of entire series so that each set of conditions would be represented by the same number of replicates as every other series selected. It should be noted that in the 1-pound dosage series the germination was consistently lower in the first germination test than in the second. This difference was caused by inadvertent drying of the sprouting seeds.

The following tabulation cites examples in table 7 in which significant germination reductions first occurred. The comparisons are between treated samples and their respective checks (L.S.D.* = 7.7):

Dosage (Lb./1000 cu. ft.)	*Exposure (Hours)*	*Moisture (Per Cent)*	*Germination Test No.*	
			First	*Second*
1	8	12	x[6]	
1	12	14		x
1	24	10	x[6]	
1	24	11	x	x
2	8	12		x
2	12	11		x
2	12	13	x	x
2	24	10	x	x
4	8	11	x	x
4	12	10	x	x
6	4	11		x
6	4	14	x	x
6	8	10	x	x
8	4	10		x
8	8	10	x	x

[6] These are questionable values because of inadvertent drying of seeds as explained in the text.

As a rule, combinations of variables having one factor of a greater value resulted in increased damage, and those with a smaller factor survived the treatment without injury. Other comparisons may be made in the table by using the appropriate L.S.D.* value. Except in the 1-pound treatment series, the delayed effect is indicated by comparing each pair (first and second) of germination tests.

Gas analyses showed that the fumigant concentrations were quite close to the calculated dosages.

Comparison with Results of Other Research with Wheat.—The results of the wheat tests differ considerably from those reported by Cotton and Frankenfeld in 1955 (unpublished data) in that their data showed that the drier seeds were more susceptible than those with 14

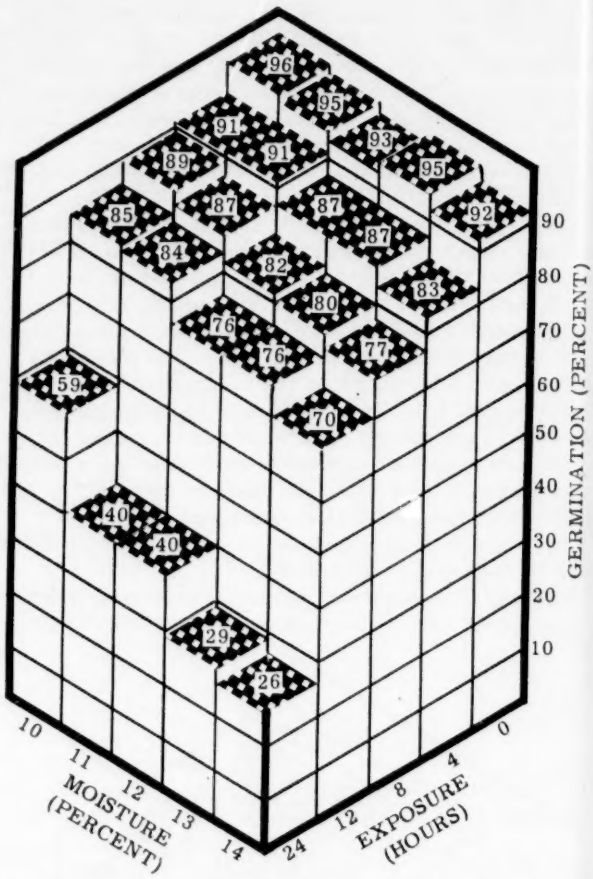


FIG. 7.—Data illustrating the interactions of seed moisture content and length of fumigant exposure on germination of Pawnee wheat 30 days after fumigation with methyl bromide, 2 lb./1000 cu. ft. at 80° F.

and 16% moisture. A contrast of the two methods of fumigation may indicate the key to the differences in results: Frankenfeld fumigated seeds in 1-quart containers fairly well loaded with wheat. The tests currently reported involved fumigations with relatively few seeds in 20-quart containers. Thus the ratio of commodity to air space was greatly different between the two experiments. It is commonly understood that the degree of fumigant sorption by the seeds is proportional to their moisture contents, *i.e.*, the higher moisture is associated with greater sorption. It is possible, therefore, that the high-moisture seeds in Frankenfeld's tests sorbed and held methyl bromide in portions of the seeds other than the embryo, and thereby reduced the effective fumigant concentration below levels toxic to the wheat embryos. If this were the case, the relatively few seeds in the tests reported here would not have had the sorptive capacity to demonstrate this theoretical phenomenon. Further research should be conducted to prove or disprove the theory because of the significant practical implications.

Fisk & Shepherd (1938) reported that a dosage of 10 pounds per 1000 cu. ft. for 5 hours' exposure was tolerated by "dry" wheat seeds. Piper & Davidson (1938) reported that fumigations which gave 100 per cent kill to 5 species of test insects (approximately 1 pound per 1000 cu. ft.

Table 6.—Pawnee wheat: effects of methyl bromide fumigation[a] on seed germination. Summary of all tests conducted.

Dosage and Length of Exposure (Hours)	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Mean Per Cent Difference[b] Between Germination[c] of Fumigated and Check Samples at Indicated Germination Test and Moisture Content												
		One Day After Fumigation					30 Days After Fumigation					6 Months After Fumigation		
		10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	12 Per Cent	14 Per Cent
0.6 lb./1000 cu. ft.														
24	11	− 1[d] (500)[f]	—[e]	1 (500)	—	0 (500)	1 (500)	—	2 (500)	—	1 (500)	—	—	—
1 lb./1000 cu. ft.														
4	16	− 4 (400)	− 6 (500)	− 6 (500)	− 4 (500)	3 (500)	2 (500)	− 1 (500)	− 5 (500)	− 3 (500)	− 8 (500)	—	—	—
8	15	− 2 (500)	− 6 (500)	−10 (500)	− 1 (400)	0 (500)	1 (500)	− 7 (500)	− 6 (500)	− 7 (500)	− 6 (500)	—	—	—
12	16	− 6 (300)	− 7 (500)	− 4 (500)	2 (500)	4 (500)	− 5 (500)	− 5 (500)	− 9 (500)	− 3 (500)	−11 (500)	—	—	—
24	16	− 3 (900)	7 (500)	− 6 (1000)	6 (500)	− 3 (1000)	− 1 (1000)	− 9 (500)	− 7 (1000)	−11 (500)	− 9 (1000)	—	—	—
2 lbs./1000 cu. ft.														
4	31	0 (2600)	− 2 (2000)	− 2 (2600)	− 1 (2000)	1 (2600)	− 4 (2600)	− 3 (2000)	− 5 (2600)	− 7 (2000)	− 9 (2600)	− 2 (600)	− 6 (600)	− 9 (600)
8	31	1 (2600)	− 3 (2000)	− 5 (2600)	− 6 (2000)	0 (2600)	− 6 (2600)	− 7 (2000)	−11 (2300)	−15 (2000)	−15 (2600)	− 3 (600)	−12 (600)	−15 (600)
12	31	− 3 (3100)	−23 (2500)	−18 (3100)	−22 (2500)	−19 (3100)	−11 (3100)	−12 (2500)	−18 (3100)	−20 (2500)	−24 (3100)	− 6 (600)	−31 (600)	−34 (600)
24	31	−32 (4100)	−57 (2500)	−56 (4100)	−70 (2500)	−62 (4100)	−39 (4100)	−58 (2500)	−57 (4100)	−60 (2500)	−72 (4100)	−41 (600)	−62 (600)	−70 (600)
3 lbs./1000 cu. ft.														
24	46	− 5 (1000)	—	−47 (1000)	—	−74 (1000)	1 (1000)	—	−41 (1000)	—	−71 (1000)	—	—	—
4 lbs./1000 cu. ft.														
4	64	0 (2100)	− 2 (1500)	− 4 (2100)	− 3 (1500)	− 6 (2100)	− 1 (2100)	0 (1500)	− 1 (2100)	− 2 (1500)	− 8 (2100)	− 3 (600)	− 8 (600)	−21 (600)
8	64	− 7 (2100)	−22 (1500)	−42 (2100)	−45 (1500)	−55 (2100)	− 8 (2100)	−19 (1500)	−36 (2100)	−44 (1500)	−53 (2100)	−10 (600)	−32 (600)	−40 (600)
12	64	−31 (2600)	−61 (2000)	−70 (2600)	−81 (2000)	−83 (2600)	−28 (2600)	−57 (2000)	−69 (2600)	−78 (2000)	−82 (2600)	−25 (600)	−59 (600)	−76 (600)
24	63	−65 (3600)	−83 (2000)	−81 (3600)	−87 (2000)	−86 (3600)	−64 (3600)	−81 (2000)	−80 (3600)	−83 (2000)	−87 (3600)	−70 (600)	−73 (600)	−73 (600)
5 lb./1000 cu. ft.														
24	82	−22 (1000)	—	−88 (1000)	—	−92 (1000)	−12 (1000)	—	−85 (1000)	—	−92 (1000)	—	—	—
6 lb./1000 cu. ft.														
4	95	−12 (1600)	− 4 (1000)	−14 (1600)	−11 (1000)	−22 (1600)	− 6 (1600)	− 6 (1000)	− 6 (1600)	− 9 (1000)	−17 (1600)	−12 (600)	−13 (600)	−46 (600)
8	99	−30 (2100)	−44 (1500)	−67 (2100)	−82 (1500)	−84 (2100)	−41 (2100)	−45 (1500)	−67 (2100)	−74 (1500)	−81 (2100)	−85 (600)	−81 (600)	−82 (600)
12	97	−66 (2100)	−79 (1500)	−84 (2100)	−87 (1500)	−89 (2100)	−66 (2100)	−80 (1500)	−83 (2100)	−86 (1500)	−88 (2100)	−76 (600)	−82 (600)	−89 (600)
24	96	−75 (3100)	−87 (1500)	−87 (3100)	−88 (1500)	−94 (3100)	−85 (3100)	−86 (1500)	−86 (3100)	−88 (1500)	−95 (3100)	−87 (600)	−86 (600)	−94 (600)
8 lb./1000 cu. ft.														
4	130	−22 (2100)	−14 (1500)	−38 (2100)	−54 (1500)	−82 (2100)	−12 (2100)	−19 (1500)	−39 (2100)	−59 (1500)	−64 (2100)	−30 (600)	−45 (600)	−68 (600)
8	128	−56 (2100)	−74 (1500)	−82 (2100)	−88 (1500)	−86 (2100)	−64 (2100)	−74 (1500)	−80 (2100)	−84 (1500)	−87 (2100)	−73 (600)	−80 (600)	−77 (600)
12	128	−77 (2100)	−84 (1500)	−85 (2100)	−87 (1500)	−92 (2100)	−78 (2100)	−84 (1500)	−84 (2100)	−88 (1500)	−93 (2100)	−77 (600)	−78 (600)	−87 (600)
24	123	−81 (3100)	−87 (1500)	−88 (3100)	−89 (1500)	−96 (3100)	−78 (3100)	−87 (1500)	−87 (3100)	−88 (1500)	−95 (3100)	−78 (600)	−79 (600)	−94 (600)

[a] The fumigation temperature was 80° F.
[b] Differences were corrected by Abbott's formula to account for dead seeds in checks.
[c] All seeds showing embryonic development were counted as germinated.
[d] Negative numbers indicate a lower value in the treated sample than in the check sample.
[e] No test made.
[f] Number of seeds tested in treated samples given in parentheses.

for 12 hours in atmospheric vault at 58° to 70° F.) had no effect on wheat viability.

In the khapra beetle research program (California State Senate 1955), preliminary test results indicated that only slight injury resulted from fumigations of wheat under these conditions: 5 lb./1000 cu. ft., 12 hours' exposure, 10% moisture, and 50° to 53° F.

The effects of repeated fumigations and of the time interval between fumigation and germination test were pointed out by Walkden & Schwitzgebel (1951). Wheat viability decreased more than 50% in association with 2 fumigations and 2 years of storage. Viability of untreated check wheat decreased only about 5%. The fumigant used was a 3 to 1 mixture (by volume) of ethylene dichloride and carbon tetrachloride containing 10% methyl bromide. Tests with the 3 to 1 mixture alone did not cause injury; in fact, it stimulated germination in some instances.

New Wheat.—A few tests were conducted with wheat about 1 month after harvest. The response of these seeds was much more erratic than for aged seeds. The tendency was for the new seeds to be more susceptible. Samples from this same lot of wheat about 3 months later responded similarly to those samples from other aged lots of wheat.

Seedling Evaluations.—Table 8 presents a summary of

Table 7.—Pawnee wheat: effects of methyl bromide fumigation[a] on seed germination. Summary and statistical statement of selected data.

Dosage and Length of Exposure (Hours)	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
		10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1 lb./1000 cu. ft.											
Untreated check	—	85.0[d]	92.3	88.3	96.3	89.7	95.0	84.3	94.3	82.7	97.7
4	16	81.2	94.0	81.6	95.2	83.8	90.4	78.6	90.8	80.8	90.2
8	15	80.8	92.6	81.8	89.4	80.8	89.2	81.4	87.0	79.0	92.2
12	16	80.6	87.0	80.8	91.4	84.8	88.0	84.2	88.8	82.6	87.4
24	16	77.2	89.2	80.6	86.6	77.2	83.8	77.0	84.2	79.8	84.0
2 lb./1000 cu. ft.											
Untreated check	—	100	98.6	98.7	96.0	98.3	97.3	99.0	99.3	99.7	93.7
4	27	99.2	96.4	99.0	97.2	99.0	93.6	99.6	97.8	99.2	94.0
8	28	98.8	95.6	98.2	96.0	97.8	89.4	97.6	93.6	98.2	95.2
12	29	96.8	98.0	94.8	87.0	94.8	89.0	84.0	87.2	87.4	86.0
24	30	63.4	66.0	26.0	27.2	29.8	27.2	23.2	24.4	29.6	18.6
4 lb./1000 cu. ft.											
Untreated check	—	97.7	97.3	99.3	98.7	94.3	95.3	98.3	95.3	94.0	96.7
4	65	97.2	96.7	96.8	96.4	96.7	96.8	98.2	96.0	91.0	90.0
8	62	93.0	95.0	81.4	75.0	62.4	47.2	66.0	37.6	24.6	21.2
12	62	85.2	89.4	32.8	27.8	15.2	10.0	7.4	8.0	5.6	4.6
24	62	10.8	15.0	6.8	5.6	5.4	5.0	6.0	4.0	2.8	0.6
6 lb./1000 cu. ft.											
Untreated check	—	99.3	96.0	98.0	95.7	98.7	96.3	99.7	93.7	97.7	96.7
4	99	95.8	92.4	94.6	83.4	94.8	88.8	93.8	83.0	82.2	70.2
8	102	91.6	83.8	66.4	61.0	32.0	21.4	18.4	11.8	7.4	5.6
12	99	55.6	38.0	20.2	10.0	9.2	8.2	8.4	8.0	3.8	2.8
24	101	4.8	7.8	5.8	5.0	4.6	4.6	4.6	4.8	1.2	2.2
8 lb./1000 cu. ft.											
Untreated check	—	98.3	93.7	98.7	86.7	98.0	89.3	97.3	96.0	97.0	87.7
4	132	94.0	78.4	85.0	44.8	70.4	32.2	56.8	11.4	17.8	5.4
8	128	42.0	12.4	12.6	8.6	5.2	4.4	5.0	5.8	5.0	2.4
12	128	9.2	5.5	6.2	6.6	5.0	5.0	3.8	3.2	1.8	0.6
24	127	6.2	4.2	7.6	5.4	5.8	3.0	3.4	3.4	0.2	0.6

[a] The fumigation temperature was 80° F. Five hundred seeds tested under each set of conditions except that 300 seeds were tested in the untreated checks.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
[d] Note that all samples in first test of the 1-lb. dosage showed lower germination than in the other tests. This was caused by inadvertent drying during incubation.
Least significant mean differences at the 5% level (L.S.D.*):
Comparing samples having 300 seeds, L.S.D.* = 8.7.
Comparing samples having 300 seeds with samples having 500 seeds, L.S.D.* = 7.7.
Comparing samples having 500 seeds, L.S.D.* = 6.7.

the tests conducted for the purpose of determining sublethal and delayed effects. The mean per cent differences in the table express corrected differences between fumigated samples and their respective checks. Only seedlings which are normal are counted as germinated by official standards (U.S. Dept. of Agriculture 1952).

Injured seeds developed more slowly than untreated seeds. This is illustrated by the fact that corrected germination differences (normal seedlings) were smaller in the 10-day counts than in the 5-day counts. This means that many of the treated seeds had abnormal sprouts after 5 days' incubation which became normal by the time of the 10-day examination. The check samples were almost completely normal after 5 days. Differences between 5-day and 10-day germination results were greatest in fumigated samples when final counts showed injuries were in the range of about 20 to 50% reduction in 10-day normal seedlings.

Pronounced increases in the percentages of abnormal seedlings are shown in table 8. These are especially notable when germinations (10-day normal seedlings) were reduced by 20 to 75% below the check samples. The abnormal seedlings appeared stunted and/or twisted with many of them lacking essential parts. Frequently the tip of the plumule failed to emerge from the coleoptile, but continued to grow in the form of a loop. As a general rule, a small number of seeds survived the most severe treatments and developed normally, indicating that a few select individuals have a high degree of tolerance to methyl bromide.

As in the other series, gas analyses at the end of each fumigation showed concentrations closely approximating the calculated amount applied.

Discussion of Results and Relation of Seed Tolerance to Dosages Required for Insect Control.—It should not be a surprise to learn that methyl bromide gas will damage or kill the seeds of our domestic plants in view of the fact that the fumigant is used as an effective means of controlling such undesirable weeds as bindweed and crabgrass. It will kill the seeds of these plants as well as the plants proper (Adamson 1956). Of course, dosages used for such purposes are considerably higher than needed for stored-grain fumigation, and a margin of tolerance exists between treatments required for insect control and those which are lethal to seeds.

It is obvious that an absolute line of demarcation can

Table 8.—Pawnee wheat: seedling evaluations to determine the effects of methyl bromide fumigation[a] on seed viability.

Dosage, Length of Exposure, and Moisture	CH_3Br Analyses (Oz./1000 Cu. Ft.)	Mean Per Cent Difference[b] between Fumigated[c] and Check[d] Samples											
		1 Day After Fumigation				30 Days After Fumigation				6 Months After Fumigation			
		5-Day Count	10-Day Count			5-Day Count	10-Day Count			5-Day Count	10-Day Count		
		Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead
2 lb./1000 cu. ft.													
4 hours	31.8												
10%		2	2	−1[e]	−1	−5	−2	0	2	1	1	−3	1
12%		−4	0	−2	2	7	19[f]	−8	−8	−15	−10	3	6
14%		−6	−2	1	2	−2	12[f]	−5	−5	−23	−25	13	9
8 hours	31.0												
10%		0	0	0	0	−2	−1	0	1	−1	−1	−3	3
12%		−17	−2	1	1	−11	−8	−1	−6	−44	−43	29	11
14%		−14	−3	1	3	−20	−4	−3	7	−56	−53	32	7
12 hours	31.0												
10%		−24	−3	2	1	−43	−14	3	10	−50	−48	39	6
12%		−46	−18	10	7	−50	−23	6	14	−55	−55	20	32
14%		−43	−19	13	6	−42	−42	12	25	−74	−73	31	34
24 hours	29.0												
10%		−61	−29	15	13	−67	−64	7	52	−71	−72	18	46
12%		−67	−56	16	38	−68	−57	−3	54	−78	−78	11	62
14%		−71	−63	21	39	−75	−74	2	69	−77	−78	2	70
4 lb./1000 cu. ft.													
4 hours	63.0												
10%		−20	−2	3	−1	−9	−7	0	7	−7	3	6	1
12%		−15	−8	2	5	−7	−8	0	7	−57	−42	31	8
14%		−25	−13	4	8	−40	−23	6	14	−66	−49	19	20
8 hours	64.0												
10%		−14	−4	0	4	−37	−8	−2	10	−62	−29	15	6
12%		−63	−37	10	25	−68	−56	21	33	−69	−62	25	32
14%		−57	−43	18	22	−71	−64	23	34	−72	−71	19	41
12 hours	62.3												
10%		−59	−28	12	15	−68	−45	19	23	−68	−67	34	25
12%		−65	−59	12	45	−76	−70	11	56	−77	−77	12	58
14%		−85	−76	1	70	−84	−82	1	76	−75	−76	−10	76
24 hours	60.0												
10%		−71	−69	1	64	−77	−76	−2	71	−74	−71	−12	69
12%		−78	−74	3	70	−81	−81	0	78	−74	−74	−4	73
14%		−88	−77	−4	77	−88	−85	−3	83	−80	−79	−6	72
6 lb./1000 cu. ft.													
4 hours	95.5												
10%		−49	−30	−1	29	−63	−38	27	10	−81	−69	55	12
12%		−53	−33	−1	31	−59	−42	30	9	−77	−71	53	13
14%		−67	−49	+1	46	−79	−72	36	27	−73	−80	25	46
8 hours	103.0												
10%		−76	−72	2	63	−75	−74	14	56	−83	−82	4	74
12%		−79	−69	−1	62	−80	−80	10	64	−77	−80	6	70
14%		−82	−79	−8	80	−89	−85	−1	78	−86	−88	0	81
12 hours	100.5												
10%		−79	−78	−4	76	−85	−84	4	77	−83	−83	3	76
12%		−78	−77	−8	77	−80	−80	−5	79	−84	−84	−1	82
14%		−86	−81	−8	81	−91	−90	−3	85	−96	−96	−1	89

[a] The fumigation temperature was 80° F.
[b] Differences were corrected by Abbott's formula to account for abnormal and dead seeds in checks.
[c] Six 100-seed samples were used for each set of conditions for the fumigated seeds.
[d] Except in a few cases, three 100-seed samples were used for each set of conditions for the check samples.
[e] Negative numbers indicate a lower value in the treated sample than in the check sample.
[f] For unknown reasons the check samples for these samples became very moldy and resulted in lower germination than the other checks or the treated samples

Table 8.—(*Continued*)

Dosage, Length of Exposure, and Moisture	CH_3Br Analyses (Oz./1,000 Cu. Ft.)	Mean Per Cent Difference[b] between Fumigated[c] and Check[d] Samples											
		1 Day After Fumigation				30 Days After Fumigation				6 Months After Fumigation			
		5-Day Count	10-Day Count			5-Day Count	10-Day Count			5-Day Count	10-Day Count		
		Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead
24 hours	97.0												
10%		−83	−82	− 9	83	−84	−85	− 3	85	−86	−86	− 4	87
12%		−80	−77	− 9	78	−87	−85	− 5	84	−90	−90	0	86
14%		−96	−90	− 5	87	−97	−96	− 4	92	−96	−97	− 4	94
8 lb./1000 cu. ft.													
4 hours	129.0												
10%		−64	−59	−33	64	−68	−65	36	24	−72	−67	27	29
12%		−75	−73	6	65	−70	−68	26	37	−78	−78	29	45
14%		−76	−76	− 3	73	−76	−74	4	61	−88	−80	1	69
8 hours	132.0												
10%		−64	−61	−43	74	−77	−75	− 2	72	−74	−74	−14	73
12%		−79	−79	− 2	78	−77	−76	− 7	76	−81	−82	− 1	80
14%		−89	−83	0	75	−90	−84	0	77	−93	−85	− 2	79
12 hours	132.0												
10%		−68	−68	−43	78	−78	−78	− 3	77	−70	−74	18	77
12%		−83	−82	− 2	82	−78	−80	− 8	81	−81	−80	− 1	78
14%		−93	−85	− 4	83	−94	−89	0	82	−98	−96	− 2	87
24 hours	125.0												
10%		−69	−71	−19	75	−78	−78	− 6	80	−77	−78	− 3	78
12%		−81	−82	− 2	82	−84	−82	− 7	82	−84	−82	0	80
14%		−98	−93	− 4	90	−98	−96	− 2	92	−100	−98	− 5	94

not be drawn to show the "safe" level of methyl bromide fumigation for insects in seeds. Rather, one should consider these factors as reported here and by others: (1) dosage, (2) moisture content of the seed, (3) length of fumigant exposure, (4) kind of seed, (5) the condition and period of storage after fumigations, (6) fumigation temperature, (7) history (previous fumigations, age, storage conditions, etc.) of the seed, (8) ratio of commodity to total space in the fumatorium (*i.e.*, sorption capacity), and (9) leakage factors in the fumatorium.

In general, for the first fumigation under carefully controlled conditions, it is possible to achieve satisfactory insect control without damage to seed viability. For example, Lindgren *et al.* (1955) reported methyl bromide LD_{95} values of 8.0 and 17.0 milligrams per liter at 70° F. for 24-hour and 8-hour exposures, respectively, for larvae of the khapra beetle (*Trogoderma granarium* (Everts)), which is considered one of the more resistant species. Dennis & Whitney (1955) found 2.7 and 5.1 milligrams per liter adequate to kill 100% of adult rice weevils (*Sitophilus oryza* (L.)) and confused flour beetles (*Tribolium confusum* Duv.), respectively, when fumigated at 70 to 80° F. for 20 hours' exposure in cylinders without the presence of a commodity. For all practical purposes milligrams per liter is equivalent to ounces per 1000 cubic foot.

Other laboratory tests by Whitney in 1956 (unpublished data) in grain-filled recirculators at 73° to 78° F. indicate that the following dosage rates are adequate to kill all stages of the rice weevil and the confused flour beetle adults, when exposed 24 hours: (1) In wheat, 10% moisture: 5 mg./l.; 12% moisture: 7.5 mg./l.; 14% moisture: 7.5 mg./l.; (2) in corn, 11.3% moisture: 7.5 mg./l.; (3) in grain sorghum, 14% moisture: 12.5 mg./l.; (4) in barley, 11% moisture: 7.5 mg./l.; and (5) in oats, 12% moisture: 7.5 mg./l. Naturally, the interstitial gas concentrations were initially higher than the calculated dosages because of displacement and soon became lower because of the sorption factor.

Thus it is shown that the minimum treatments necessary for insect control may be used without expectation of injury to seeds. The margin of tolerance, however, may be quite narrow, and such factors as repeated fumigations and low initial viability may result in unexpected damage. Further testing is in progress.

References Cited

Adamson, R. M. 1956. Control of field bindweed with methyl bromide. Down to Earth **12**(13): 12.

California State Senate. 1955. Special report of the joint interim committee on agricultural and livestock problems . . . on the khapra beetle, *Trogoderma granarium*. Senate of California. 106 p.

Cotton, R. T., J. C. Frankenfeld, E. G. Bayfield, and J. A. Johnson. 1946. Wheat viability loss due to fumigation and its relation to baking quality. Milling Production 11(7): 8–9.

Dennis, N. M., and W. K. Whitney. 1955. Effect of varying the volatilization of methyl bromide by combinations with various solvents on its distribution in bulk grain fumigation. U. S. Dept. Agric., Agr. Mktg. Serv. **AMS-73,** 18 p.

Fisk, Frank W., and Harold H. Shepherd. 1938. Laboratory

studies of methyl bromide as an insect fumigant. Jour. Econ. Ent. 31(1): 79–85.

Hlynka, K., and J. A. Anderson. 1949. A comparative study of ten electrical meters for determining moisture content of wheat. Canadian Jour. Res. **27F:** 382–97.

Lindgren, David L., Lloyd E. Vincent, and H. E. Krohne. 1955. The khapra beetle, *Trogoderma granarium* Everts. Hilgardia **24**(1): 21–6.

Mackie, D. B. 1938. Methyl bromide—its expectancy as a fumigant. Jour. Econ. Ent. **31**(1): 70–9.

Monro, H. A. U., C. T. Buckland, and J. E. King. 1953. Preliminary observations on the use of the thermal conductivity method for the measurement of methyl bromide concentrations in ship fumigation. Ent. Soc. Ontario Ann. Rept. **84:** 71–6.

Phillips, G. L. 1957. Current use of thermal conductivity gas analyzers for the measurement of fumigants. Pest Control **25**(7): 18, 20–2, 24 and 26.

Phillips, G. L., and J. W. Bulger. 1953. Analysis of methyl bromide by measurements of thermal conductivity. U. S. Dept. Agric., Bur. Ent. and Plant Quar. **E-851.** 7 p.

Piper, W. R., Jr., and R. H. Davidson. 1938. Methyl bromide vapor against five species of stored product insects. Jour. Econ. Ent. **31**(3): 460–1.

U. S. Dept. of Agriculture. 1952. Testing agricultural and vegetable seeds. U. S. Dept. Agric., Agriculture Handbk. No. **30.** 440 p.

Walkden, H. H., and R. B. Schwitzgebel. 1951. Evaluation of fumigants for control of insects attacking wheat and corn in steel bins. U. S. Dept. Agric., Tech. Bull. **1045.** 20 p.

Bioassay of the Blood from Cattle Treated with Am. Cyanamid 12,880[1]

R. H. ROBERTS,[2] R. D. RADELEFF,[3] and J. N. KAPLANIS,[2] *Agr. Res. Serv., U.S.D.A.*

ABSTRACT

This systemic compound, known chemically as *O,O*-dimethyl S-(N-methylcarbamoylmethyl)phosphorodithioate (also called dimethoate) was administered to cattle by oral, intramuscular, and intravenous routes. Blood was taken from animals at stated intervals following treatment and bioassayed with the stable fly (*Stomoxys calcitrans* (L.)) as the test organism. The blood levels determined by radiometric measurement of P^{32}-labeled 12,800 were lower than those determined by bioassay. An unknown metabolite(s) in the blood was found to be approximately 40 times as toxic as the parent compound to stable flies. Am. Cyanamid 12,880 and the metabolites toxic to stable flies were not detectable by the procedure after 24 hours. The toxic materials were detectable for a longer period when the parent compound was administered orally than when it was given by intramuscular or intravenous routes.

In conjunction with research on systemic insecticides at the Kerrville, Texas, laboratory, a program using biological assay methods was initiated. Its purpose was to help correlate research data obtained by other workers at the laboratory and to determine the insecticidal activity of the metabolites found in the blood or waste products or the amount of insecticide present in the blood after treatment as compared with that found by other methods. The use of bioassay as a tool for the determination of residues is particularly important with new materials for which quantitative chemical procedures have not been standardized.

This paper reports tests to determine levels of Am. Cyanamid 12,880 (*O,O*-dimethyl S-(N-methylcarbamoylmethyl)phosphorodithioate) (also called dimethoate) in the blood following various routes of administration.

MATERIAL AND METHODS.—Am. Cyanamid 12,880 either labeled with P^{32} or unlabeled, was administered to yearling cattle by oral, intramuscular, and intravenous routes. The compound was given orally as a drench or in gelatin capsules. The drench was prepared by diluting a 30%-alcohol solution with water to make a 1.5% solution. For intramuscular injection a 50-50 (wt./wt.) mixture with diethyl succinate was used. For intravenous administration a 10%-alcohol solution was diluted with 300 ml. of a 0.9%-saline solution and injected by means of a Simplex kit for 3 to 5 minutes. Table 1 gives the treatment schedule.

The blood from the treated animals was heparinized to prevent clotting and bioassayed, using two replicates of 60 to 70 starved 3- to 6-day-old stable flies (*Stomoxys calcitrans* (L.)) of both sexes. The blood was prepared by dilution with fresh heparinized blood from a Hereford steer, and then placed in homeopathic vials 6×1.5 cm., with cotton wicks. The flies fed readily on the blood absorbed by the wick and, except for an occasional straggler, feeding was completed within the first 15 minutes. Mortality readings were made 24 hours later, and the per cent mortality was corrected by Abbott's formula.

The LD-50 for Am. Cyanamid 12,880 previously determined by adding known amounts of the blood was 1.7 p.p.m., and the LD-90 was 2.8 p.p.m. The parts per million of toxic materials present, expressed as 12,880, were read directly from the regression line on the log-probit graph.

At various times after treatment with the labeled material, the blood of the animals was plated, and radiometric measurements were made to determine the levels of 12,880. The blood samples were extracted with an organic solvent, hexane-dichloromethane, and the organo-soluble materials were measured radiometrically after being chromatographically separated. The extracted materials were also tested by bioassay.

RESULTS.—The levels of Am. Cyanamid 12,880 found in the blood of the animals following the various routes of administration are presented in tables 2 to 6. The toxic materials were detectable for a longer period when the parent compound was administered orally than when it was given by intramuscular or intravenous routes.

The first 24-hour blood samples from Animal V were pooled and extracted. The extract contained a total of 21.7 micrograms of two materials, the parent compound

[1] Accepted for publication June 19, 1958.

[2] Entomology Research Division.

[3] Animal Disease and Parasite Research Division.

Table 1.—Schedule for treatment of cattle with Am. Cyanamid 12,800.

Animal No.[a]	Weight (Kg.)	Dosage (Mg./Kg.)	Route
	Unlabeled compound		
I	229	3	Intravenous
II	125	3	do.
III	260	15	Oral; drench
	Labeled compound		
IV	175	3	Intravenous
V	147	15	Oral; capsule
VI	128	10	Intramuscular
VII	186	10	Oral; capsule
VIII	194	10	Intramuscular
IX	130	10	Intravenous

[a] All Hereford steers except III, which was an Angus cross heifer, and IV which was a Hereford heifer.

and an unknown metabolite(s) in the ratio of 2 to 1. Another extract from the 3- and 6-hour samples yielded 8.1 micrograms, of which 20.1% was the unknown compound and 79.9% was 12,880. A small amount, 1.7 micrograms, of the unknown was obtained by elutriation from the chromatographic strips. The mortalities of stable flies fed various concentrations of these extracts are shown in table 7. The extract from the 3- and 6-hour blood samples was stored for 7 weeks at 2° C. When retested at the end of this period at the level originally causing 94% mortality, there was no significant mortality. Similarly, the extract of the unknown metabolite(s) alone failed to cause significant mortality when tested after 3 weeks' storage.

Extracts of the unknown metabolite (s) from the blood of Animal VII also demonstrated this loss of toxicity with time. When first tested at 0.04 p.p.m. 4 days after extraction there was no significant mortality. At 0.07 p.p.m. after 6 days there was 66% and at 0.09 p.p.m. after 10 days there was 63% mortality.

The toxic materials present in the blood were eliminated over a 24-hour period. In one animal they were not completely eliminated until after 30 hours. During this period of decline, the ratio of the parent compound to the toxic metabolite(s) changed. As the percentage of the 12,880 decreased, the percentage of its unknown analog increased. Table 8 gives the per cent of 12,880 in the extract of the blood samples.

Table 2.—Am. Cyanamid 12,880 in blood of three animals treated intravenously with 3 mg./kg. (Parts per million.)

Time After Treatment	Bioassay			Total Radioactivity
	I	II	IV	IV
0	9.6	12.8	12.4	9.88
10 minutes	5.4	10.0	9.2	7.11
20	3.2	11.6	11.9	6.11
40	4.6	11.6	17.8	5.17
60	3.2	11.6	12.4	4.11
2 hours	0.9	7.8	3.4	2.75
3	—[a]	—	0.9	1.86
4	0.9	2.5	1.1	1.45
6	N.S.[b]	0.8	N.S.	0.83
9	—	—	N.S.	0.67
12	—	—	N.S.	0.24
24	N.S.	0.8	N.S.	Trace

[a] Not assayed.
[b] No significant mortality.

Table 3.—Am. Cyanamid 12,880 in blood of two animals treated orally with 15 mg./kg. (Parts per million.)

Hours After Treatment	Animals		Radiometric Measurements, V	
	III	V	Total	Organo-soluble Compounds
½	10.2	—	—	—
1	12.8	47.3	8.81	3.28
2	20.8	—	—	—
3	24.4	84.0	13.5	3.88
4	23.8	—	—	—
5	21.6	—	—	—
6	18.0	102.0	11.6	2.84
7	12.8	—	—	—
8	11.0	—	—	—
9	8.8	82.8	8.7	1.41
12	2.7	49.6	5.7	0.77
18	—	22.8	3.05	0.14
24	N.S.[a]	8.8	1.85	0.07

[a] No significant mortality.

Autopsies were performed on Animals V, VI, VII, and VIII. Animals VI and VII were negative for pathological changes.

Animal V, which was severely poisoned at the dosage of 15 mg./kg., revealed on gross examination of the kidneys severe degenerative changes of the cortical regions. This animal is believed to have been suffering from this kidney malfunction prior to treatment, which may have aggravated the symptoms of poisoning. The dosage employed is known to have caused mild poisoning in healthy cattle of the same age.

Animal VIII presented a number of abnormal and pathological conditions at autopsy. These consisted of multiple abscesses of the liver 2.5–5.0 cm. in diameter; a small infarcted area in the cortex of the right kidney; a diffuse pleuritis with a few adhesions of the lungs to the chest wall and chronic interstitial lobular pneumonia; a mild pericarditis with an accumulation of approximately 50 ml. of fluid mixed with fibrin strands; and a congenital deformity of the brain, in which the cerebral hemispheres were filled with fluid leaving a thin sac approximately 1 cm. thick.

Table 4.—Am. Cyanamid 12,880 in blood of two animals treated intramuscularly with 10 mg./kg. (Parts per million.)

Hours After Treatment	Bioassay		Radiometric Measurements: Total		Radiometric Measurements: Organo-soluble Compounds	
	VI	VIII	VI	VIII	VI	VII
1	27.0	18.0	12.3	10.12	7.24	4.91
3	29.0	13.6	10.35	5.91	3.48	1.65
6	25.2	6.0	5.53	2.26	1.02	0.41
9	15.3	N.S.[a]	3.10	0.97	0.19	0.25
12	7.6	N.S.	1.90	0.46	0.07	Trace
18	1.4	N.S.	0.88	0.11	Trace	0
24	N.S.	N.S.	0.42	0.06	Trace	0

[a] No significant mortality.

Table 5.—Am. Cyanamid 12,880 in blood of one animal (VII) treated orally with 10 mg./kg. (Parts per million.)

Hours After Treatment	Bioassay	Radiometric Measurements	
		Total	Organosoluble Compounds
1	3.6	3.09	0.36
3	8.0	4.34	0.74
6	9.6	5.32	1.01
9	10.1	4.35	0.89
12	14.4	2.80	0.68
18	9.1	1.83	0.39
24	4.2	1.00	0.18
30	1.6	0.5	0.05

Table 7.—Bioassay results with an unknown metabolite(s) alone and mixed with the parent compound (Am. Cyanamid 12,880).

Parts per Million		Per Cent Mortality
Unknown(s)	12,880	
0.2	0.4	100
.15	.3	100
.1	.2	99
.07	.14	87
.05	.2	94
.05	—	69
.04	.16	90
.04	—	10
.03	.06	10

DISCUSSION.—Am. Cyanamid 12,880 is metabolized and removed from the blood within 24 hours after treatment. It appears to be removed more rapidly when given by the intravenous and intramuscular routes. The slower absorption into the system when given by the oral route would account for the presence of toxic materials in the blood for a longer period.

The data from Animal V, even though it was pathologically abnormal, explain to some degree the pattern of level of insecticide in the blood of animals treated intravenously (tables 2 and 6). After the intravenous introduction of 3 mg./kg., which would give a theoretical level of 20 to 30 p.p.m. if all the material stayed in the blood, there is a drop after 10 minutes followed by a rise to a peak or a plateau during the first hour, after which the toxic materials disappear. This pattern would be caused by the removal of the parent material from the blood followed by the production of metabolites as the compound is broken down. One or more of these metabolites are toxic, causing the rise noted above. These materials are in turn further metabolized and lose their toxic properties.

This pattern is less evident in the animals treated by the intramuscular and oral routes. In these animals there is a gradual increase as the material is absorbed into the system. The rate and the amount of toxic metabolites produced vary from animal to animal. The presence of some metabolite that is more toxic than the parent compound is evident when the levels obtained biologically and radiometrically are compared. It will be noticed that the levels of toxic materials, expressed as 12,880, determined by bioassay are greater, particularly when compared with the levels obtained from the blood by extraction with an organic solvent. This unknown(s) may well be one of the first metabolites formed, since even at zero time the animal treated intravenously had a higher level by bioassay than by radiometric measurements of the unextracted blood.

A few tests conducted with the unknown metabolite(s) showed a toxicity to stable flies nearly 40 times as great as the parent compound. The levels at which these tests were run were below the level detectable for 12,880 alone. In addition, this metabolite appeared to have a different toxicity when tested in different proportions with the 12,880. This material also breaks down in storage after several weeks. At present the identity of this metabolite can only be surmised. Since it appears so early after administration of 12,880 to the animal, it could be the oxygen analog of the parent compound.

The autopsy conditions given above for Animals V and VIII are believed to have been present before treatment, and not due to the effect of the chemical. The kidney damage in Animal V probably caused the high levels in the blood by slowing down the rate of excretion.

CONCLUSIONS.—1. Am. Cyanamid 12,880 and its metabolites toxic to stable flies are removed from the

Table 6.—Am. Cyanamid 12,880 in blood of one animal (IX) treated intravenously with 10 mg./kg. (Parts per million.)

Time After Treatment	Bioassay	Radiometric Measurements	
		Total	Organosoluble Compounds
0	50.8	25.45	13.25
10 minutes	66.9	21.60	9.88
20	51.7	19.25	8.21
40	62.0	15.95	6.06
60	55.9	13.75	3.61
2 hours	51.2	9.70	1.90
3	56.0	7.73	0.94
4	34.0	5.78	0.43
6	21.6	4.79	0.16
9	11.2	2.61	0.04
12	3.4	1.48	Trace
24	N.S.[a]	0.29	0

[a] No significant mortality.

Table 8.—Per cent of Am. Cyanamid 12,880 present in the organo-soluble extracts of the blood from animals treated by three routes of administration.

Time After Treatment	Animals		
	VII	VIII	IX
0	—	—	97.6
10 minutes	—	—	96.5
20	—	—	96.2
40	—	—	90.8
60	93.1	97.8	89.0
2 hours	—	—	84.1
3	86.7	90.1	73.3
4	—	—	32.3
6	83.6	51.4	—
9	83.7	32.7	—
12	77.2	—	—
18	74.8	—	—
24	62.2	—	—

blood within 24 hours after treatment. They are removed sooner when the treatment is intravenous or intramuscular than by the oral route.

2. As determined by radiometric measurement, the parts per million of the toxic materials decrease steadily from the initial treatment level but as determined by bioassay as 12,880, they first increase to a peak and then decrease steadily.

3. The peak noticed in the biological determinations is apparently caused by the production of one or more metabolites of unknown chemical composition. The unknown(s) was approximately 40 times more toxic than the parent compound.

4. The ratio of the unknown metabolite to the parent compound increased steadily during the period when they are found in the blood.

5. The mortality readings are expressed as parts per million of 12,880 although actually one or more of the unknown metabolites cause most of the toxic action against stable flies.

An Evaluation of the Physical Characteristics and Biological Effectiveness of Plastic Coated Glass Fiber Insect Screens[1]

LAFE R. EDMUNDS, ROBERT G. HAHL, and CARL WESLEY, JR., *Engineer Research and Development Laboratories, Fort Belvoir, Virginia*

ABSTRACT

Five samples of plastic coated glass fiber insect screening materials ranging in weight from 2.5 to 5.0 ounces per square yard were studied to evaluate their physical characteristics and biological effectiveness. Average bursting strength, in Mullen Points, ranged from 147 to 235. When wet the samples lost 34 to 84% of their strength; however, when allowed to dry for 2 days they recovered 82 to 100% of their initial strength. When exposed at 165° F. for 6 months, three samples gained in strength; one was unchanged and one lost strength. After 24 hours at −65° F., two samples failed while three samples showed no evidence of cracking or flaking. Natural weathering tests showed that the screening materials did not corrode, and held up well under a variety of conditions.

Tests on resistance to insect attack showed that the plastic coated glass fiber insect screens were very resistant to most species. Of the test insects, only crickets chewed openings in the screens. Resistance to mildew growth was satisfactory in all but one sample. Screens of 18×18 mesh excluded the majority of insects and still permitted enough air to circulate for satisfactory ventilation. Screens of coarser mesh were unsatisfactory. Insecticides applied directly to the screens did not harm them. Tests under the direction of personnel from the Engineer Research and Development Laboratories, Fort Belvoir, Virginia, were made from 1950 through 1957 and are summarized in this paper.

Screens are used on buildings to protect humans from flies, mosquitoes, and other insect pests or vectors of disease. Screens for excluding pests and disease-bearing insects are recognized as essential in providing health protection for personnel at our military bases around the world. It is important that these screening materials be strong, weather resistant, and insect proof.

Numerous complaints were received over the years in regard to premature failure of the plastic and metal insect screening materials in use. Plastic screening materials either shrank, frequently breaking or bowing screen frames, or expanded to present an unsightly baggy appearance. Some metal screening materials disintegrated within 6 months because of excessive corrosion action, and some left disfiguring stains on window sills, door frames, and sides of buildings.

In preliminary tests a number of different commercially available screening materials were evaluated. These included copper, brass, bronze, galvanized steel, saran, and plastic coated glass fiber. The plastic coated glass fiber screens showed promise and they were given additional physical and biological tests.

MATERIALS AND METHODS.—*Materials.*—Samples of five different plastic coated fibrous glass screening materials were tested. The mesh per inch on the samples was 18×14 or 18×18. The glass yarn had a diameter of 11 mils.

Physical Methods.—The materials were tested for (1) weight, (2) bursting strength, (3) effects of soap solution, (4) heat aging, (5) accelerated weathering, (6) blocking, (7) resistance to heat of burning cigarettes, (8) low temperature embrittlement, (9) natural weathering, (10) effects of insecticide solutions.

Biological Methods.—The materials were also tested for (1) resistance to insect attack, (2) mildew resistance, (3) insect penetration.

PHYSICAL TESTS AND RESULTS.—*Weight.*—The weight per square yard was determined at 70° F. and 65% relative humidity. The five samples of plastic coated fibrous glass screens ranged from 2.5 to 5.0 ounces per square yard (table 1). Comparable samples of metal screens weighed from 20 to 36.8 ounces per square yard.

Bursting Strength.—Bursting strength is a measure of the force necessary to produce a rupture of a material where pressure is applied at a controlled increasing rate. The bursting strength is measured in Mullen Points; one Mullen Point is approximately equivalent to 1 pound per square inch. Tests were performed at 70° F. and 50% relative humidity. Using a standard 0.035-inch diaphragm the average bursting strength of the insect screen in Mullen Points ranged from 147 to 235 (table 1).

Wet Bursting Strength and Recovery.—Samples of screen were immersed for 7 days in distilled water at 73.5° F. Mullen bursting tests were made while the

[1] Accepted for publication June 27, 1958

Table 1.—Results of physical tests on five samples of plastic coated glass fiber insect screen.

MESH SIZE	OZ. PER SQ. YARD	BURSTING STRENGTH (MULLEN POINTS)[a]	WET BURSTING STRENGTH (MULLEN POINTS)[b]	PERCENTAGE OF INITIAL STRENGTH RECOVERY[c]	BURSTING STRENGTH AFTER IMMERSION IN SOAP[d]	PER CENT LOSS OF STRENGTH AFTER 240 HOURS' WEATHERING	BLOCKING AT 3 P.S.I.
18×14	4.9	235	155	96	152	42	None
18×18	2.5	147	49	82	65	24	Blocks
18×18	3.5	235	38	No test samples	No test samples	No test	None
18×14	5.0	205	97	85	132	32	None
18×14	5.0	215	169	100	175	38	None

[a] One Mullen Point is approximately equivalent to 1 pound per square inch (0.035-inch diaphram).
[b] Tested while wet after seven days' immersion in distilled water.
[c] Seven days' immersion in distilled water followed by drying for two days at 73.5° F.
[d] Tested while wet after 24-hour immersion in 1% soap solution.

screen was still wet. The loss in strength averaged from 34 to 84%. When the screen was immersed for 7 days and allowed to dry for 2 days at 73.5° F. and 50% relative humidity, the samples recovered from 82 to 100% of their initial strength (table 1).

Effects of Soap Solutions.—Samples of screen were immersed 24 hours in a 1% solution of Army soap dissolved in distilled water. Mullen bursting tests were run immediately while the screen was still wet. The bursting strength ranged from 65 to 175 (table 1).

Heat Aging.—Samples of screens were suspended in a forced air oven at 165° F. for 6 months. At the end of this period, the samples were tested by means of the Mullen Tester, using the 0.076-inch diaphragm. Three samples gained in strength, one sample remained unchanged, and one sample lost strength (table 2).

Accelerated Weathering.—In an attempt to determine quantitatively the loss of strength due to accelerated weathering, samples of screens were placed in a weatherometer for 240 hours. Thereafter, Mullen bursting tests were run and the results of three tests were averaged. The samples lost 24% or more of their original bursting strength.

Blocking.—Blocking is the adhesion between touching layers of material such as occurs under moderate pressures during storage or use. Blocking tests were performed using a temperature of 165° F. and pressure of 3 p.s.i. for a period of 24 hours. No blocking occurred with four samples, one sample failed (table 1).

Low Temperature Embrittlement.—Samples of screen were tested for low temperature embrittlement by conditioning at −65° F. for 24 hours, then forcing 4-inch square samples through a 0.306-inch orifice at −65° F. The samples were brought to room temperature and examined visually for evidence of cracking or flaking of the plastic coating or yarn. Two samples failed while three samples showed no evidence of cracking or flaking (table 2).

Resistance to Heat of Burning Cigarettes.—Samples of screen were tested for resistance to the heat of a burning cigarette. The samples were burned in two locations with the lighted end of a cigarette, for periods of 10 seconds. In this procedure, the cigarette was held in position vertically, with no pressure applied other than the weight of the cigarette. The plastic burned or charred, but the glass yarns remained intact on all five samples (table 2).

Flame Resistance.—Flame resistance was determined by subjecting a 2×12 inch sample, held vertically, to the flame of a lighted match. The flame was placed in direct contact with the base of the sample for 10 seconds. The flame source was removed and the time that the specimen continued to burn was noted. Tests were run in triplicate, then averaged. The average time interval the screen samples continued to burn after removal of the flame ranged from 0 to 14 seconds (table 2).

Natural Weathering.—Natural weathering tests were performed in different parts of the world in order to expose the screens to all types of weather conditions. These tests were made in a mild but changeable climate at Fort Belvoir, Virginia; in the Arctic at Fort Churchill, Canada (fig. 1); in the desert at Yuma, Arizona; in salt air exposed at Miami Beach, Florida; and in the tropics at Fort Sherman, Canal Zone. All screens were installed in 2×5 foot frames, so that duplicates could be exposed at each test site. Tests were made at each site for 8 months during 1952. This information is summarized in table 3. Since the plastic coating on the glass fibers has a tendency to char or burn when exposed to the lighted end of a cigarette, a study was made on the effects of natural weathering on a burned area. The burned areas withstood weathering very well (table 3). The screens were left exposed at all sites from 1952 through 1957 with no appreciable change caused by weathering.

Effects of Insecticide Solutions.—Coated fiber glass insect screens at all of the weathering stations were painted

Table 2.—Results of flame and temperature tests on plastic coated glass fiber insect screens.

PER CENT CHANGE IN BURSTING STRENGTH BY HEAT AGING[a]	LOW TEMPERATURE TESTS	RESISTANCE TO BURNING CIGARETTE[b]	AVERAGE TIME SCREENS CONTINUED TO BURN AFTER FLAME REMOVAL[c] (SECONDS)
0	No damage	Yarns intact	14.0
+21	Failed	Yarns intact	4.3
−21	Failed	Yarns intact	8.3
+11	No damage	Yarns intact	4.0
+ 2	No damage	Yarns intact	0.0

[a] Six months' exposure in forced air oven at 165° F.
[b] Lighted cigarette touched yarn for 10 seconds.
[c] The flame was placed in direct contact with sample for 10 seconds, flame source was removed and time specimen continued to burn was noted.

FIG. 1.—Natural weathering tests of plastic coated glass fiber insect screens exposed in 2-×5-foot frames at Fort Churchill, Canada from 1952 through 1957.

with four different formulations of DDT; 5% DDT in kerosene, in xylene, in water, and 5% DDT-2% chlordane. Monthly inspections were made and it was concluded by Wesley & Morrill (1956), that the insecticides used had no harmful effects on the samples tested.

BIOLOGICAL TESTS AND RESULTS.—*Resistance to Insect Attack.*—The plastic coated glass fiber insect screens were tested for resistance to insect attack. This work was done by R. H. Nelson and P. G. Piquett of the U. S. Department of Agriculture at the request of the Engineer Research and Development Laboratories. The work was reported in Department of Agriculture Interim Report No. C 81, March 25, 1953.

The following insects were placed inside cages made of the plastic coated fiber insect screen or in battery jars containing such cages: *Blattella germanica* (L.), *Periplaneta americana* (L.), *Blatta orientalis* (L.), *Acheta assimilis* F., *Melanoplus* spp., *Tenebriodes mauritanicus* (L.).

None of the cockroaches or grasshoppers chewed through the screen. The field crickets chewed through the screen in all combinations. Larvae of the cadelle squeezed

Table 3.—Results of 8 months' exposure of plastic coated insect screens to natural weathering at five different sites.[a]

Location of Test Site[b]			
Yuma, Arizona	Miami Beach, Florida	Fort Sherman, Panama	Fort Belvoir, Virginia
Color fades to grey. Some slackness. Burned areas discolored	No change	Slight bulging	Uniform vertical streaks. Loss of plastic exposed glass fibers
Color fades to grey. Increased slackness	Uniform fading. Loss of plastic in burned area	White spots.[c] Increased slackness	Color faded. Loss of plastic in burned areas
Sand adherence. Some slackness	Uniform fading. Loss of plastic in burned areas	1 screen bulged. Otherwise no change	No change
Color fades to grey. Vertical streaks. Dirt film. Wrinkling along edge	No change	Light colored[c] spots. Some bulging	Uniform vertical streaks. Slight loss of plastic in burned areas
Some wrinkling and slackness. Some fading in burned areas	Burned area became darker in color	Slight bulging	Slight loss of plastic in burned area. Slight color change

[a] In preparation for weathering tests the screening samples were burned with cigarettes in certain areas.
[b] No change in any of the samples at Fort Churchill, Canada was noted.
[c] Probably fungi.

through the screen interstices but did not damage the screen. The cadelle adults did not penetrate the screen.

Mildew Resistance.—Five samples of plastic coated glass fiber insect screen were tested for resistance to mildew (fungus growth). One sample of each screen was tested in a non-nutrient agar medium allowing 21 days for incubation. Two samples showed no mildew while three showed slight to moderate mildew. A second sample of each screen was tested in non-nutrient agar medium and checked visually every 7 days. Two samples showed no mildew while three showed some growth. A third sample of each screen was suspended in a tropical test chamber for 6 months. The test chamber was operated on a daily temperature-humidity cycle comparable to tropical areas. All of the samples had mildewed slightly. One sample failed the test and was heavily mildewed.

Insect Penetration.—Insect penetration of various mesh screens was tested using *Aedes aegypti* mosquitoes (Wesley & Morrill 1956). No penetration of insects through meshes finer than 18×18 occurred, screens of mesh coarser than 18×18 were not satisfactory in restricting the mosquitoes. It was found that screens of 18×18 mesh would exclude the majority of insects and still permit enough air for satisfactory circulation.

DISCUSSION.—Plastic coated glass fiber insect screening material has certain advantages over the materials previously used. Some of the important advantages are: resistance to corrosion; does not stain window sills or outside walls; has dimensional stability; is easily installed and is lightweight (Osborn & Myers 1953). The raw materials used to manufacture this type of screen are readily available in wartime.

Wesley & Morrill (1956) applied solutions of 5% DDT (in kerosene, in xylene, as wettable powders, and with 2% chlordane) directly onto the screens under test. The plastic on the screens did not soften or dissolve nor did it appear to be harmed in any other way.

Results from the experiments on the air and insect penetration of insect screens, to determine the most effective size, resulted in the recommendation that only 18×18 mesh be procured for military use. It was concluded that screens of 18×18 mesh should be used because this mesh excluded the majority of insects and yet permitted enough air for pleasant ventilation.

As a form of amusement, soldiers sometimes punctured saran and nylon screens with lighted cigarettes. When cigarettes were touched against the plastic coated insect screens, the plastic coating burned or charred but the glass yarns remained intact.

Laboratory weathering, and biological tests demonstrated that plastic coated glass fiber insect screening material was satisfactory for military use. As a result of these studies a specification was prepared describing a screen having the best characteristics of the screens studied. This specification L-S-137 for *Screening, Plastic-Coated Fibrous Glass, Insect,* is now being used to procure screening for military use.

Besides being of value to the Military Forces, the information obtained on screening materials is useful in a civilian capacity for more effective exclusion of pest and disease bearing insects and preventing the disfigurement of buildings that results when metal screens corrode.

ACKNOWLEDGMENTS.—The authors are particularly indebted to the following people for assistance in making these tests: Mr. Harry N. Lowe, Jr., Chief of the Sanitary Engineering Branch, Mr. James M. Osborn and Mr. Frederick Myers, Materials Branch, Engineer Research and Development Laboratories; also Mr. R. H. Nelson and Mr. P. G. Piquett of the Department of Agriculture. Thanks are extended to Corps of Engineers personnel at various test sites for their valuable aid.

REFERENCES CITED

Nelson, R. H., and P. G. Piquett. 1953. Insect attack on plastic coated screen. Department of Agriculture Interim Report No. **C 81,** 61 pp.

Osborn, J. M., and F. C. Myers. 1953. Plastic coated glass fiber insect screens. Engineer Research and Development Laboratories Report. **1327.**

Wesley, C., Jr., and A. W. Morrill, Jr. 1956. Air and insect penetration of insect screens. Mosquito News **16**(3): 204–6.

Wesley, C., Jr., and A. W. Morrill, Jr. 1956. Effects of various insecticide solutions on different kinds of insect screens. Mosquito News **16**(3): 206–8.

Dehydrogenase Systems from Fat Body of Susceptible and Chlordane Resistant *Blattella germanica* (L.)[1]

ROGER G. YOUNG,[2] *Department of Entomology, Cornell University, Ithaca, N. Y.*

ABSTRACT

The presence and levels of diphosphopyridine nucleotide malic dehydrogenase and triphosphopyridine nucleotide glucose-6-phosphate and 6-phosphogluconate dehydrogenases have been established in the fat body of a susceptible and a chlordane-resistant strain of cockroach (*Blattella germanica* (L.)). The method used was spectrophotometric measurement of the enzymatically reduced nucleotides. A statistically significant difference between male insects of the susceptible and resistant strains was found for malic dehydrogenase. It is not known how the difference is related to resistance.

This report is concerned with the presence of some of the coenzyme-linked dehydrogenases in the fat body of susceptible and chlordane-resistant German cockroaches, *Blattella germanica* (L.).

There are numerous reports (Sacktor 1950, Pratt & Babers 1953, Fullmer & Hoskins 1951) of comparisons of terminal oxidative metabolism in susceptible and insecti-

[1] Accepted for publication June 30, 1958.
[2] Grateful acknowledgment is made to Millicent S. Ficken for assistance.

Table 1.—Dehydrogenase activities of *Blattella germanica* fat body.[a]

Substrate	Normal Cockroaches		Resistant Cockroaches	
	Males	Females	Males	Females
DPN-Malate	26.2 ±3.5[b] (16)[c]	34.4 ±4.1 (23)	37.6 ±4.0 (17)	41.4 ±5.0 (27)
TPN-G6P	.93± .18 (10)	1.29± .40 (11)	.87± .23 (11)	.72± .57 (9)
TPN-6 PG	3.54± .47 (11)	4.15± .44 (13)	3.81± .69 (10)	3.69± .52 (9)

[a] Moles DPN or TPN reduced per hour per kg. protein.
[b] S.E. of mean.
[c] The figures in parentheses indicate the number of insects tested. The greater number of malic dehydrogenase assays result from expansion of the program to include TPN dependent enzymes.

cide resistant insects. The purpose of this work was to establish the presence of the three main energy producing metabolic pathways at the substrate level in the fat body of the German cockroach and to determine whether differences exist between a susceptible and a resistant strain.

A variety of dehydrogenases requiring diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN) as coenzymes has been investigated in insect tissues. Chefurka (1955) demonstrated the presence of the TPN linked dehydrogenases which constitute the initial stages of the glucose phosphate shunt in house flies. Faulkner (1956) studied TPN coupled enzyme systems in silkworm blood which utilized malate and sugar phosphates as substrates. A DPN specific α-glycerophosphate dehydrogenase is present in house fly thoracic sarcoplasm, (Sacktor & Cochran 1957). A malic dehydrogenase whose coenzyme is DPN has been shown to be present in the fat body of the American cockroach, *Periplaneta americana*, by Young (1958).

Materials and Methods.—*Experimental Insects.*—Susceptible and chlordane resistant strains of German cockroaches were obtained from Dr. James M. Grayson of the Virginia Polytechnic Institute. They were reared in 19-quart polyethylene waste baskets on pulverized dog food and water. The resistant characteristic of the one strain was maintained by exposure of colonies to chlordane impregnated filter paper for 3 days every 5 weeks. After exposure, the living gravid females were killed. Only fourth and fifth nymphal stages were used for the experiments.

DPN Malic Dehydrogenase.—The fat body of individual insects was dissected and immediately frozen and dried under vacuum. Ten to 30 microgram portions of the dried material were weighed on a Salvioni balance (sensitivity 0.1 μg.), transferred to a tube and assayed immediately. The reaction was carried out by the method of Strominger & Lowry (1955). The mixture consisted of the fat body, 100 μl. of a mixture of 0.02 M malate and 0.1 M glycine buffer at pH 10.2 and 0.2 M DPN.[3] After mixing, optical density readings were made at a wave length of 340 mμ in a Beckman DU spectrophotometer fitted with an adapter block for 0.5 cm. I.D. tubes as described by Lowry & Bessey (1946). Time intervals of 2 to 20 minutes were sufficient to give reduction of DPN corresponding to maximum optical density increases of 0.200. Incubation was at room temperature, 25 to 30° C. Each insect was assayed in triplicate. Although the equilibrium constant for this enzyme favors malate formation (Stern *et al.* 1952), we have found that the rate measured with malate as the substrate at pH 10.2 was one-half that with oxalacetate as substrate.

TPN Dehydrogenases.—The tissue was removed and immediately homogenized in 0.2 ml. of cold 0.04 M glycyl glycine buffer, pH 7.4. The reaction mixture at pH 7.4 in a total volume of 150 μl. contained 10 μl. homogenate, 1 μM Mg Cl_2, 1.0 μM substrate under test, 0.16-μM TPN[3], and 0.8 μM glycyl glycine. After mixing, the optical density change was followed at 340 mμ to measure reduced TPN. Five to 30 minutes incubation times were sufficient to show optical density changes not exceeding 0.400 units. Temperature and replication were as for malic dehydrogenase. Controls containing all components except substrate were run. Substrates used were 6-phosphogluconate[3] (1.0 μM for 6-phosphogluconate dehydrogenase), and mixtures of 6-phosphogluconate and glucose-6-phosphate[3], 1.0 μM each. The glucose-6-phosphate dehydrogenase activity was then calculated by difference.

Protein.—This was determined on trichloroacetic acid insoluble material by the method of Lowry *et al.* (1951).

Results.—The results are summarized in table 1. The specificity of malic dehydrogenase was tested by replacement of DPN by TPN. No reduction of TPN occurred in 25 minutes with an enzyme preparation which reduced DPN at the rate of 0.077 optical density units per minute.

Discussion.—A rough comparison of the data of Chefurka (1955) with that reported here indicates that fat body contains several times the TPN reducing activity of house fly muscle. It is also noteworthy that the fat body contains a considerable preponderance of 6-phosphogluconate dehydrogenase over glucose-6-phosphate dehydrogenase rather than the reverse as for house flies.

The finding of up to 60-fold greater malic dehydrogenase activity than the hexose phosphate type may indicate the greater importance of the tricarboxylic acid cycle in the fat body, although the pentose pathway has been suggested to be of some importance in insects (Newburgh & Cheldelin 1955).

The use of a t-test for unequal numbers, to compare the values of appropriate pairs of data indicates only that in male insects is there a significant (5% level) difference between the malic dehydrogenase levels in chlordane resistant and susceptible groups of insects. A similar study of the glycolytic activity of the same tissues by

[3] Purchased from Sigma Chemical Co., St. Louis, Mo.

means of assays for aldolase showed no difference between strains (Young, unpublished results).

That the oxidative metabolism of resistant strains of insects may differ from susceptible ones has been demonstrated by studies of oxygen uptake (Pratt & Babers 1953), and cytochrome oxidase (Perry & Sacktor 1955) in susceptible and DDT-resistant house flies. In neither study were differences between susceptible and resistant strains consistent through all the possible comparisons of susceptible and resistant strains.

The fact that a significant difference in malic dehydrogenase in females was not found here would suggest that no relationship between the enzyme and resistance was possible. In view of the fact that markedly different quantitative manifestations of resistance between sexes of the same insect are known, *e.g.*, susceptibility to chlordane and order of magnitude of resistance (Grayson 1954), it does not seem impossible that sex differences in defense mechanisms against insecticides exist.

A decrease in the standard error of mean values of enzyme assays of insect populations by knowledge of the exact stage of development at the time of assay has been achieved (Young, unpublished data). This will facilitate strain and sex comparisons and eventual resolution of the above question.

There is some other indirect evidence that the energy producing mechanisms may be factors in resistance to insecticides. Wigglesworth (1956) has suggested that resistance to DDT may result from maintenance of active transport of ions maintained in the presence of insecticide by the oxidation systems of the nerve sheath. The report of Winteringham *et al.* (1958) that dieldrin-resistant strains of flies excrete the insecticide more rapidly would argue for intact or enhanced endergonic reaction sequences for driving detoxication processes in the resistant strains. A study of detoxifying enzyme relationships with oxidative metabolism is in progress.

REFERENCES CITED

Chefurka, W. 1955. The occurrence of a direct oxidative pathway of carbohydrate metabolism in the fly *Musca domestica* L. Biochim. et Biophys. Acta **17**: 295–6.

Faulkner, W. 1956. Enzymic reduction of sugar phosphates in insect blood. Biochem. Jour. **64**: 436–41.

Fullmer, O. H., and W. M. Hoskins. 1951. The effects of DDT upon the respiration of susceptible and resistant house flies. Jour. Econ. Ent. **44**: 858–70.

Grayson, J. M. 1954. Differences between a resistant and a non-resistant strain of German Cockroach. Jour. Econ. Ent. **47**: 253–6.

Lowry, O. H., and O. A. Bessey. 1946. The adaptation of the Beckman spectrophotometer to measurements on minute quantities of materials. Jour. Biol. Chem. **163**: 633–9.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. Jour. Biol. Chem. **193**: 265–75.

Newburgh, R. W., and V. H. Cheldelin. 1955. Oxidation of carbohydrates by the pea aphid, *Macrosiphum pisi* (Kltb.). Jour. Biol. Chem. **214**: 37–42.

Perry, A. S., and B. Sacktor. 1955. Detoxification of DDT in relation to cytochrome oxidase activity in resistant and susceptible house flies. Ann. Ent. Soc. America **48**: 329.

Pratt, J. J., and F. N. Babers. 1953. The resistance of insects to insecticides. Jour. Econ. Ent. **46**: 864–9.

Sacktor, B. 1950. A comparison of cytochrome oxidase activity of two strains of house flies. Jour. Econ. Ent. **43**: 832–8.

Sacktor, B., and D. G. Cochran. 1957. DPN specific α-glycerophosphate dehydrogenase in insect flight muscle. Biochim. et Biophys. Acta **25**: 649–50.

Stern, J. R., S. Ochoa, and F. Lynen. 1952. Enzymatic synthesis of citric acid. V. Reaction of Acetyl Coenzyme A. Jour. Biol. Chem. **198**: 313–21.

Strominger, J. L., and O. H. Lowry. 1955. Quantitative histochemistry of the brain. IV. Lactic, malic, and glutamic dehydrogenases. Jour. Biol. Chem. **213**: 635–46.

Wigglesworth, V. B. 1956. Insect physiology in relation to insecticides. Jour. Roy. Soc. Arts, April 27, 426–37.

Winteringham, F. P. W., G. T. Brooks, A. Harrison, M. A. McKay, and A. Weatherby. 1957. WHO Information Circular on Resistance Problem 11: 1.

Young, R. G. 1958. Adolase and malic dehydrogenase in the fat body of *Periplaneta americana*. Proc. Tenth Internatl. Congr. Ent. (In Press).

Methyl Bromide Fumigation against European Chafer in Nursery Stock[1]

H. TASHIRO, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

ABSTRACT

Eggs, first- and third-instar larvae, pupae, and adults of the European chafer, *Amphimallon majalis* (Raz.), infesting soil balls 10 inches in diameter were exposed for 2½ hours to methyl bromide in an enclosed chamber. Third-instar larvae were completely eliminated with 7, 5, 4, 2, and 2 pounds at 40, 45, 50, 60 and 70° F., respectively. In exposures of other stages to 1 to 4 pounds per cubic feet at 70 to 74° F., complete elimination of first-instar larvae and adults was obtained with the 1-pound dosage and eggs and pupae with 3 pounds.

Atmospheric fumigation with methyl bromide in a tight chamber is authorized by the Plant Pest Control Division (1958) for the elimination of all stages of the Japanese beetle, *Popillia japonica* Newm., in the soil about roots of plants to permit their movement under quarantine regulations. Experiments were conducted during 1956 to determine the effectiveness of this treatment in eliminating the soil-inhabiting stages of the European chafer, *Amphimallon majalis* (Raz.), in nursery stock for the same purpose. Any treatment short of complete eradication is considered inadequate for quarantine requirements. Therefore, studies were aimed at

[1] Accepted for publication July 7, 1958.

[2] In cooperation with the Department of Entomology, New York State Agricultural Experiment Station, Geneva, N. Y.

determining temperature-dosage combinations that would meet this requirement.

FUMIGATION CHAMBER.—Tests were conducted in a 100-cubic-foot fumigation chamber in which the air temperature was controlled thermostatically within 3° F. This permitted a variation of only 2° in the soil temperature because of its slower heat conductivity. A circulating fan dispersed the gas and kept the temperature uniform throughout the chamber.

Methyl bromide was measured into a plastic flask in the closed feed-line assembly between the supply cylinder and the chamber. Rapid vaporization was obtained by passing it through a coil of ¼-inch copper tubing surrounded by a hot-water jacket on its way to the chamber. At the end of the fumigation period the chamber was aerated. An exhaust fan with a capacity of 125 cubic feet per minute was mounted in the stack.

The chamber was relatively tight according to the standards set up by Chisholm & Koblitsky (1945). Tightness, measured by increasing the air pressure within the chamber and determining the time required for it to return to atmospheric pressure, indicated that it was equivalent to that of a 500-cubic foot chamber having a maximum opening of ⅜ inch. Further inspection by checking all joints and points of possible leaks with a halide lamp during the fumigation period indicated very little leakage.

PROCEDURES.—Artificial soil balls 10 inches in diameter and 10 inches high were prepared with moist Ontario loam that had been passed through ½-inch-mesh screen and seeded with domestic rye grass. The soil was compressed into cylinders made of ½-inch-mesh hardware cloth lined with burlap. To obtain the same degree of compaction as found in the field, several balls of similar size, moisture, and soil type obtained in the field were weighed. The mean weight of these balls, 42½ pounds, was used as the basis for compacting the artificial balls.

As each soil ball was being prepared it was infested with the chafer in one of its stages. Six balls were prepared for each treatment, five to be fumigated and one for a check. Those to be fumigated were held overnight in the closed chamber under controlled temperature. When necessary a thermometer was placed in the center of a soil ball and checked before fumigation to ascertain that the required temperature had been obtained.

When the desired air and soil temperatures were obtained, the methyl bromide was introduced into the chamber with the circulating fan in operation. After an exposure of 2½ hours, the chamber was exhausted and aerated for at least 30 minutes before it was unloaded.

After fumigation, the soil balls were usually held for a week; then the live chafers were transferred to untreated soil and held at room temperature for further observation. Most stages were examined at half-week intervals for 3 weeks and discarded after an examination at the end of 4 weeks.

All stages were included in the experiments, but the most extensive tests were conducted against third-instar larvae, since it is the stage present for the longest period—up to 9 months of the year, in the fall, winter, and spring—and constitutes the greatest hazard of dissemination in plant material.

In tests with third-instar larvae 50 individuals were distributed uniformly in four layers in each soil ball. Each larva was placed in a ½-inch depression made with a dibble to serve as a cell and prevent the larva from being crushed during compression of the soil above it. Each ball was held for a week to allow the larvae to adjust themselves to their environment. The first fumigation treatments were made at 40° F. As the temperature was raised the dosage of fumigant was lowered. When the soil balls were broken down 1 week after fumigation, it was found that the larvae treated at 40° tended to migrate downward, but there were a few throughout the balls. In soil balls treated at higher temperatures the larvae were fairly well distributed. All the live larvae recovered from each ball were placed in a tray of moist untreated soil for further observation.

In tests with pupae 24 soil balls were infested with 50 third-instar larvae per ball as previously described and then placed in a compact group outdoors and occasionally sprinkled to maintain soil moisture. Fumigation treatments were started when most of the larvae had transformed to pupae. The few larvae and prepupae present in the soil balls when broken down were disregarded in the evaluations. Since it was impossible to determine whether crushed pupae were alive or dead, they were also disregarded. All live pupae were placed in shallow depressions on the surface of moist untreated soil in trays for further observation. Since recently dead pupae were difficult to determine, only those that had turned black through partial decomposition were regarded as being dead.

In tests with adults, groups of 25 males and 25 females were placed in small screen cages, and the cages filled loosely with soil. A cage of each sex was placed in the center of each soil ball and the soil around them firmly tamped. The next day they were fumigated. Because of the short life span of adults, soil balls were broken down 3 days after fumigation.

In tests with eggs field-collected gravid females were held in moist soil for oviposition. Groups of 50 well-developed eggs were placed in single-layer cheesecloth bags with soil. Each bag was inserted in a roll ofwindow screen for protection against crushing and placed in the center of a soil ball. The next day the soil balls were fumigated. The eggs were then removed from each ball and placed in depressions on the soil surface in a petri dish to be observed at semiweekly intervals for hatching. First-instar larvae were removed from the dishes as soon as observed. At the termination of the tests 4 weeks after breakdown of the balls, the soil in each dish was sifted to recover any of the larvae that had migrated into the soil.

In tests with first-instar larvae 4-inch clay pots filled with moist soil and germinating red top seed were each infested with 50 well-developed eggs. The pots were plunged in soil and held until all the eggs had hatched. Then the surface of each pot was covered with a layer of cheesecloth, secured with rubber bands, to keep the soil in the pot separate from the soil in the balls. One pot was buried near the center of each soil ball. The next day the soil balls were fumigated. A week later they were torn down and the soil from each pot was screened to recover the larvae. All live individuals were transferred to untreated soil for further observation.

RESULTS.—The results of these tests are summarized

Table 1.—Mortality of third-instar European chafer larvae exposed to various dosages of methyl bromide for 2½ hours at different soil temperatures.

Temperature (° F.)	Methyl Bromide (Pounds per 1000 Cu. Ft.)	Per Cent Mortality in— 1 Week	2 Weeks	3 Weeks	4 Weeks
40	4	16	47	55	61
	5	98	99	99	99
	6	80	89	91	92
	7	98	100	—	—
45	4	73	89	95	97
	5	98	100	—	—
	6	100	—	—	—
	7	100	—	—	—
50	3	89	98	98	98
	4	100	—	—	—
	5	100	—	—	—
	6	100	—	—	—
60	2	95	100	—	—
	3	100	—	—	—
	4	100	—	—	—
	5	100	—	—	—
70	1	8	28	38	45
	2	100	—	—	—
	3	100	—	—	—
	4	100	—	—	—
Checks[a]	0	5	10	13	16

[a] Mean results for all temperatures tested.

in tables 1 and 2. Fumigation with methyl bromide for 2½ hours was effective in killing third-instar larvae at temperatures ranging from 40° to 70° F. with dosages decreasing from 7 pounds per 1000 cubic feet at the lowest temperature to 2 pounds at the highest temperature. At 70° 1 pound killed all first-instar larvae and adults, but 3 pounds were required to eliminate eggs and pupae.

Between 50° and 70° F. 1 pound of methyl bromide per

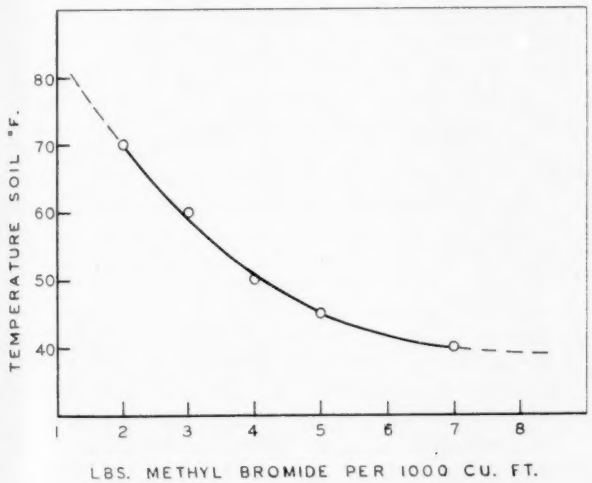


FIG. 1—Temperature-dosage requirements for elimination of third-instar European chafer larvae with 2½ hours' exposure to methyl bromide chamber fumigation.

Table 2.—Mortality of different stages of the European chafer exposed to methyl bromide for 2½ hours at soil tempertatures of 70° to 74° F.

Methyl Bromide (Pounds per 1000 Cu. Ft.)	Per Cent Mortality In 7 Days: Eggs	First Instars	Pupae	Adults in 3 Days
1	34	100	21	100
2	99	100	91	100
3	100	100	100	100
4	100	100	100	100
Check	0	1	8	56

1000 cubic feet against third-instar larvae had the same effect as 10° of temperature. Below 50° an additional pound was required to produce the same results with a 5° drop and 3 additional pounds to offset a 10° drop in temperature. Extrapolation of the curve of minimum effective dosages as shown in figure 1 indicated the expectancy of complete elimination with a 1½--pound dosage at 75° and with 1⅓ pounds at 80°. Increased dosages could not be expected to compensate for decreases in temperature below 40°. The position of the larvae in the soil ball had no influence on the effectiveness of the treatments, an indication that there was good penetration of the toxicant throughout the soil balls.

The mean recovery of 21 pupae per soil ball (table 2) was judged to be satisfactory, considering the high natural mortality during the prepupal and pupal periods. All fumigated pupae were unable to transform to adults, whereas practically all untreated pupae did become adults. There was no indication of sexual difference in susceptibility of adults to methyl bromide. There was a high mortality of untreated adults. Examination after 4 weeks showed that all viable eggs had hatched during the 1-week holding period following fumigation. The recovery in the checks of 34.3 first-instar larvae from 50 eggs was considered to be normal.

Swank & Latta (1950) indicated that in their tests against white-fringed beetle larvae most broadleaf evergreens tolerated the recommended dosage schedules ranging from 6 pounds of methyl bromide per 1000 cubic feet under vacuum at 40° to 3 pounds at 70° F., but the coniferous evergreens were injured to some degree. Judging from these results, 3 pounds per 1000 cubic feet at 70° would be a safe treatment for elimination of all stages of the European chafer in soil about the roots of most broadleaf nursery plants.

REFERENCES CITED

Chisholm, R. D., and L. Koblitsky. 1945. A method of rating fumigation chambers for tightness. U. S. Bur. Ent. and Plant Quar. **ET-224,** 4 pp.

Plant Pest Control Division. 1958. Manual of approved treatment procedures to be used under the Japanese beetle quarantine. U. S. Dept. Agric., Agric. Res. Serv., 30 pp.

Swank, G. R., and R. Latta. 1950. Vacuum fumigation with methyl bromide to kill larvae of white-fringed beetles. Jour. Econ. Ent. **43**(1): 25–9.

Field Tests with the Systemic Insecticides Thimet and Bayer 19639 as Cottonseed Treatments in 1957[1]

C. R. Parencia, Jr., C. B. Cowan, Jr., and J. W. Davis, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

Abstract

Thimet at 1 pound and Bayer 19639 at 1, 1.5, and 2 pounds per acre applied to reginned cottonseed reduced the stand of cotton significantly below that obtained from untreated seed. Thimet at 1 pound per acre did not reduce the stand when applied to acid- or flame-delinted seed. When Thimet-treated seed was planted in the drills in which a previous such planting was made, severe phytotoxicity occurred. Plants recovered but fruiting was delayed. However, yields were not reduced because conditions were favorable for plants to set fruit later in the season. The seed treatments gave thrips control for 4 weeks after plant emergence. Thimet at 1 pound per acre held cotton fleahopper infestations below injurious levels 4 weeks after plant emergence when applied to reginned seed but not when applied to acid-delinted seed. Thimet was slightly more effective than Bayer 19639 against this pest.

The effectiveness of various systemic insecticides as cottonseed treatments against early-season cotton insects in field experiments conducted near Waco, Tex., in 1954, 1955, and 1956 has been reported (Parencia *et al.* 1957a, b). It is the purpose of this paper to discuss results obtained against thrips (*Frankliniella* sp.), the cotton aphid (*Aphis gossypii* Glov.), the cotton fleahopper (*Psallus seriatus* (Reut.)), the boll weevil (*Anthonomus grandis* Boh.), and the desert spider mite (*Tetranychus desertorum* Banks) with Thimet and Bayer 19639 in additional field tests conducted in 1957.

Procedure.—One experiment was conducted in small plots and three were in large plots. The small plots were 16 rows wide and of sufficient length for an area of 0.2 acre. They were arranged in randomized blocks with four replications of each treatment. The large plots ranged in size from 1 to 6 acres, and there were no replications.

Methods of applying the materials to cottonseed and of planting were the same as those previously reported. Reginned seed was used in experiment 1, but treated flame- and acid-delinted seeds were also used in some plots of the other experiments. A conventional spray treatment—toxaphene applied at 0.75 pound per acre at weekly intervals for 4 or 5 weeks to plants grown from untreated seed—was included in each experiment.

Effect on Plant Emergence.—In experiments 1, 2, and 4 treated and untreated seeds were planted in mid-April, but 20 inches of rain between April 19 and May 12 resulted in failure to obtain stands. Seed carrying the same treatments was planted in the same drills as soon as the soil dried enough to permit this operation. One planting was made in experiment 3, but owing to wet soil conditions which made planter adjustment difficult, only about one-half of the desired planting rate was used. Results are shown in table 1.

In experiment 1 Thimet and Bayer 19639 on reginned seed reduced the stand significantly below that obtained from untreated seed. In experiment 2 Thimet on reginned seed seriously reduced the stand, but stands from the same treatment of acid- or flame-delinted seed were not affected. Since a high percentage of injured seed coats due to close reginning was found in this lot of reginned seed, this condition may have permitted the compound to enter the seed and thus affect germination. In experiment 3, in which seed that was not so closely reginned was planted, there was no difference in stand obtained from treated and untreated seed when differences in planting rates were considered. In experiment 4 there was some reduction in stand from Thimet on reginned seed.

Phytotoxicity.—In previous years there was some injury to cotyledons from the insecticides, but phytotoxicity to true leaves was minor. In 1957 in experiments 1 and 2, in which treated seed was planted in the same drills of the initial plantings, severe phytotoxicity to true leaves occurred from the Thimet treatments and lesser injury in experiment 4. There was no injury in experiment 3. In experiment 1 ten days after plant emergence 20 plants pulled at random from each plot were weighed in the laboratory. Those collected from the Thimet plots weighed significantly less than those from untreated or Bayer 19639 plots. In experiment 2 plant samples from Thimet-treated acid-delinted, flame-delinted, or reginned seed plots weighed less than those from untreated seed plots. Differences in plant appearance (size and vigor) were very noticeable for several weeks.

Since there was no severe injury to true leaves in experiments conducted at this laboratory in previous years, or in experiment 3 this year in which only one planting was made, it was concluded that this phytotoxicity probably resulted from the Thimet remaining in the drill from the initial planting.

Effectiveness Against Thrips.—Results against thrips are shown in table 2. In experiment 1 all seed treatments gave good thrips control for 3 weeks after plant emergence. After 4 weeks there was considerable loss in effectiveness, but even then infestations were about half that in the check. Only Thimet was significantly better than the toxaphene spray treatment, although the infestation in the sprayed plot was also significantly below that of the check. Further thrips records could not be made, because beginning at that time all plots had to be protected from bollworms with the toxaphene-DDT spray mixture.

In experiment 2 Thimet at 1 pound per acre applied to acid-delinted, flame-delinted, and reginned seed gave as good control as toxaphene spray 4 weeks after plant emergence. There was considerable loss of effectiveness at that time in a treated acid-delinted seed plot planted at a rate to give 0.5 pound of Thimet per acre. All seed treatments lost effectiveness after 5 weeks.

In experiment 3 after 4 weeks both Bayer 19639 dosages and Thimet on acid-delinted seed lost effectiveness, but Thimet on reginned seed continued to give fair

[1] Accepted for publication July 10, 1958. Presented at the meeting of the Southwestern Branch, Entomological Society of America, at Houston, Texas, February 10–11, 1958.

[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Planting and plant-emergence records in field experiments with Thimet and Bayer 16939 employed as cottonseed treatments.

INSECTICIDE (POUNDS PER ACRE)	POUNDS OF SEED PER ACRE	NUMBER OF SEEDLINGS PER 100 FEET OF ROW	
Experiment 1 (Planted June 11)[a]			
		June 18	
Thimet 1	23.2	261	
Bayer 19639 1	22.9	242	
1.5	23.1	240	
2	23.1	222	
Toxaphene spray	23.9	326	
Untreated check	23.9	324	
L.S.D. at 5% level	—	50	
Experiment 2 (Planted May 28)			
		June 8	*June 18*
Thimet 1[b]	28.8	321	413
1[c]	32.6	374	433
1[a]	28.7	228	215
0.5[c]	16.8	285	264
Toxaphene spray[a]	29.0	264	366
Untreated check[a]	29.0	356	377
Experiment 3 (Planted May 28)			
		June 8	*June 17*
Thimet 0.5[a]	13.7	303	293
0.5[c]	12.0	311	323
Bayer 19639 0.6[a]	16.2	301	332
1.2[a]	15.1	275	274
Toxaphene spray[a]	19.7	384	388
Untreated check[a]	20.9	351	342
Experiment 4 (Planted June 11)			
		June 18	
Thimet 1.3[c]	33.3	481	
0.9[a]	23.2	252	
.5[c]	12.0	201	
.7[a]	17.7	175	
Toxaphene spray[a]	23.9	404	
Untreated check[a]	23.9	299	

[a] Reginned seed.
[b] Flame-delinted seed.
[c] Acid-delinted seed.

control. Toxaphene spray gave poorer control than is usually expected, probably because of greater exposure to thrips migration since the plot was located on the field edge.

In experiment 4 the higher Thimet dosage on acid-delinted seed and both dosages on reginned seed gave good control for 4 weeks after plant emergence. The 0.5-pound dosage on acid-delinted seed was considerably less effective. As in experiment 1, the necessity of protecting the plants from bollworms made it impossible for further thrips records to be made.

EFFECTIVENESS AGAINST COTTON APHID AND COTTON FLEAHOPPER.—Results are given in table 3. In experiment 1, 4 weeks after plant emergence cotton aphid populations were significantly lower in the Thimet and Bayer 19639 treated-seed plots than in the untreated-seed or toxaphene-sprayed plots, although they were below injurious levels in all plots. Results were similar in experiments 2, 3, and 4. In experiments 1 and 4 the plots received 10 applications of toxaphene-DDT spray at 5-day intervals for bollworm (*Heliothis zea* (Boddie)) control between July 17 and August 26. Aphid infestations failed to develop appreciably in treated- or untreated-seed plots after treatment for bollworm control.

Only in experiments 2 and 3 were cotton fleahoppers sufficiently abundant for an evaluation of the seed treatments. In experiment 2 four weeks after plant emergence injurious infestations occurred in both the plots planted with Thimet-treated acid-delinted seed, but they were below such levels in the treated reginned or flame-delinted seed plots. Infestations continued to be lower in these plots after 5 weeks, although they had increased to injurious levels. In experiment 3 after 4 weeks infestations were lower in the Thimet than in the Bayer 19639 treated-seed plots. After 5 weeks they increased to injurious levels in all treated-seed plots. Both the treated-seed and sprayed plots of both experiments were then sprayed with toxaphene at 0.75 pound per acre for this control.

LATE-SEASON INSECTS.—Records on boll weevil and bollworm infestations are given in table 4. Boll weevil infestations were too low to be of significance, but bollworms were a factor in all experiments. All plots in experiment 1 and all except the check in experiment 4 were treated with 10 spray applications of 2 pounds of toxaphene plus 1 pound of DDT per acre between July 17 and August 26 for this control. In experiment 2 the toxaphene-sprayed plot received seven applications of 2 pounds of toxaphene plus 1 pound of DDT per acre, and the seed-treated plots seven applications of 0.4 pound of endrin between July 16 and August 13. In experiment 3 four applications of the same insecticides were made in similar plots between July 17 and 31.

In experiment 1 a few desert spider mites were found in the untreated-seed plots 7 weeks after plant emergence.

Table 2.—Effectiveness of Thimet and Bayer 19639 as cottonseed treatments against thrips.

INSECTICIDE (POUNDS PER ACRE)	NUMBER OF THRIPS PER PLANT AFTER—WEEKS INDICATED					
	1	2	3	4	5	Average
Experiment 1[a]						
Thimet 1	0.01	0.1	0.8	3.5	—	1.1
Bayer 19639 1	.01	.5	1.2	3.1	—	1.2
1.5	.06	.6	2.0	4.6	—	1.8
2	.08	.5	1.1	4.1	—	1.4
Toxaphene spray 0.75	1.26	1.8	1.8	2.9	—	1.9
Untreated check	2.62	9.4	6.0	8.2	—	6.5
L.S.D. at 5% level	.22	1.2	1.4	2.2	—	.7
Experiment 2						
Thimet 1[b]	0	0.05	0.2	2.2	4.5	1.4
1[c]	0	0	.6	2.1	4.2	1.4
1[a]	0	0	.02	2.0	7.8	2.0
0.5[c]	0	.2	.4	5.8	8.2	2.9
Toxaphene spray 0.75[a]	.7	1.8	1.1	2.2	3.2	1.8
Untreated check[a]	3.4	3.8	7.4	7.7	10.4	6.5
Experiment 3						
Thimet 0.5[a]	0	0	0.4	4.5	7.4	2.5
.5[c]	.02	.3	.9	7.5	11.4	4.0
Bayer 19639 0.6[a]	.05	.2	.6	6.5	8.9	3.2
1.2[a]	0	.1	.6	6.2	11.8	3.8
Toxaphene spray 0.75[a]	4.8	5.8	2.2	5.3	8.5	5.3
Untreated check[a]	2.5	16.4	6.0	16.7	21.0	12.5
Experiment 4						
Thimet 1.3[c]	0.05	0.2	1.1	1.8	—	0.8
0.9[a]	.02	.1	.5	1.5	—	.5
.5[c]	.05	.5	2.5	4.0	—	1.8
.7[a]	0	.2	2.4	1.5	—	1.0
Toxaphene spray 0.75[a]	2.1	1.9	2.2	1.8	—	2.0
Untreated check[a]	8.9	10.4	6.6	5.9	—	7.9

[a] Reginned seed.
[b] Flame-delinted seed.
[c] Acid-delinted seed.

Table 3.—Effectiveness of Thimet and Bayer 19639 as cottonseed treatments against the cotton fleahopper and cotton aphid 4 to 5 weeks after plant emergence.

Insecticide (Pounds per Acre)	Number of Aphids per Plant After—		Number of Fleahoppers per 100 Terminals After—	
	4 Weeks	5 Weeks	4 Weeks	5 Weeks
Experiment 1[a]				
Thimet 1	0.04	—	4.0	—
Bayer 19639 1	.05	—	4.8	—
1.5	.18	—	9.5	—
2	.02	—	9.5	—
Toxaphene spray 0.75	1.9	—	7.5	—
Untreated check	1.8	—	10.0	—
L.S.D. at 5% level	1.1	—	3.7	—
Experiment 2				
Thimet 1[b]	0.2	0.2	11.3	35.0
1[c]	.5	.5	29.6	47.0
1[a]	.02	0	14.0	30.7
0.5[c]	.8	.7	32.0	53.4
Toxaphene spray 0.75[a]	4.7	2.0	18.0	21.7
Untreated check[a]	1.6	.3	23.0	71.0
Experiment 3				
Thimet 0.5[a]	0.4	0.1	6.4	30.7
.5[c]	0	.2	5.0	35.0
Bayer 19639 0.6[a]	.05	0	12.4	41.6
1.2[a]	0	.02	9.3	38.0
Toxaphene spray 0.75[a]	1.4	.2	2.3	14.4
Untreated check[a]	.6	.4	11.4	42.0
Experiment 4				
Thimet 1.3[c]	0	—	8.3	—
0.9[a]	.02	—	6.0	—
.5[c]	.5	—	4.7	—
.7[a]	.02	—	9.3	—
Toxaphene spray 0.75[a]	8.9	—	3.3	—
Untreated check[a]	2.1	—	13.0	—

[a] Reginned seed.
[b] Flame-delinted seed.
[c] Acid-delinted seed.

After 9 weeks a few were found in the Thimet-treated-seed plots, but none in those planted with Bayer 19639-treated seed. However, infestations were too light to determine the effective period of the seed treatments against this pest.

EFFECT ON FRUITING AND YIELDS.—Fruiting as indicated by bloom records is shown in table 5, and yields are given in table 4.

In experiment 1 the plants recovered from Thimet injury, but there was a definite delay in fruiting. Since the bollworm treatment, which also gave thrips control, was begun 4 weeks after plant emergence, loss of effectiveness against thrips of the seed treatments at that time, as indicated in table 1, could have had little effect on fruiting. Since the untreated-seed plots also received this treatment, valid comparisons of fruiting could be made. On July 30 there were significantly fewer blooms in all the treated-seed plots than in the toxaphene-sprayed and untreated- seed plots. However, on August 2 there were significantly fewer blooms in the Thimet-treated than in the Bayer 19639-treated-seed plots and the untreated-seed plots, with no difference between the latter treatments. Blooming continued at considerably lower levels in the Thimet-treated-seed plots through August 9, and did not reach the same level with the other plots until August 20, when their blooming peak had passed.

This experiment was located on land with good moisture-holding capacity, and plants in the Thimet plots were able to continue fruiting, setting a crop of bolls later in the season. These plots could be readily distinguished from the others for nearly 2 weeks by the presence of blooms after plants in the other plots had ceased blooming. Delay in fruiting was further demonstrated by open-boll records. On September 27 there were significantly fewer open bolls in the Thimet-treated than in all other plots. Only 33% of the bolls were open in this plot, whereas in the other plots the percentage ranged from 62 to 81. Yield records obtained on December 4 showed no significant difference between treatments. However, under less favorable moisture conditions, which frequently occur in the area, the delay in fruiting could result in a reduced yield.

In experiment 2 the toxaphene-sprayed and all treated-seed plots received treatment for control of cotton fleahopper 5 weeks after plant emergence, and bollworm treatments were begun after 6 weeks. The check received none of these treatments, and plants in it had more blooms on July 18, 22, and 26 than those in the plots planted with treated seed, although the difference in the Thimet on reginned seed was slight on the two last dates. On July 30 all treated-seed plots had surpassed the check in blooming. These records clearly showed that the seed treatment was responsible for a delay in fruiting. Fewer blooms in the later records in the Thimet-treated acid-delinted seed plots than in the other seed-treated plots

Table 4.—Late-season boll weevil and bollworm infestations, and yields in plots in which Thimet and Bayer 16939 were used as cottonseed treatments.

Insecticide (Pounds per Acre)	Seasonal Average Infestation (Per Cent)			Pounds of Seed Cotton per Acre	
	Boll Weevil	Bollworm		Total	Gain Over Check
		Squares	Bolls		
Experiment 1[a]					
Thimet 1	0	5.0	1.8	1111	17
Bayer 19639 1	.2	4.9	1.2	1159	65
1.5	.4	5.3	1.7	1096	2
2	.4	5.9	1.0	1098	4
Toxaphene spray 0.75	.3	5.0	2.0	1063	−31
Untreated check	.2	6.0	2.5	1094	—
Experiment 2					
Thimet 1[b]	3.6	4.2	3.4	961	271
1[c]	4.7	4.0	3.0	1172	482
1[a]	2.3	4.1	4.8	1194	504
0.5[c]	3.2	4.5	3.8	1113	423
Toxaphene spray 0.75[a]	2.7	5.7	4.2	942	252
Untreated check[a]	4.6	4.9	6.1	690	—
Experiment 3					
Thimet 0.5[a]	1.8	2.7	2.5	968	423
0.5[c]	1.9	2.1	1.2	721	176
Bayer 19639 0.6[a]	2.5	2.1	2.9	918	373
1.2[a]	2.2	2.3	1.9	1000	455
Toxaphene spray 0.75[a]	2.1	4.1	4.0	979	434
Untreated check[a]	3.7	3.5	2.9	545	—
Experiment 4					
Thimet 1.3[c]	0	6.8	3.0	1463	453
.9[a]	.2	6.6	2.6	1274	264
.5[c]	0	5.2	3.5	1259	249
.7[a]	0	8.1	2.3	1568	558
Toxaphene spray 0.75[a]	0	7.4	2.5	1244	234
Untreated check[a]	0	8.2	7.4	1010	—

[a] Reginned seed.
[b] Flame-delinted seed.
[c] Acid-delinted seed.

Table 5.—Bloom counts in cotton fields in which Thimet and Bayer 19639 were employed as seed treatments, Waco, Tex., 1957.

INSECTICIDE (POUNDS PER ACRE)	NUMBER OF BLOOMS PER ACRE							
	Experiment 1 (Planted June 11)[a]							
	July 30	*Aug. 2*	*Aug. 6*	*Aug. 9*	*Aug. 12*	*Aug. 15*	*Aug. 20*	*Ave.*
Thimet 1	70	1,165	4,000	6,990	9,645	11,550	11,545	6,424
Bayer 19639 1	600	3,315	7,860	11,480	14,270	14,220	10,750	8,928
1.5	620	3,290	6,700	9,120	12,560	13,965	12,420	8,382
2	605	2,965	6,855	10,185	12,875	14,075	10,520	8,297
Toxaphene spray 0.75	1,445	4,645	10,760	14,510	15,455	16,820	8,230	10,266
Untreated check	1,300	4,040	7,810	11,160	14,185	16,075	11,415	9,426
L.S.D. at 5% level	567	1,436	2,261	2,845	2,661	1,672	—	1,423
	Experiment 2 (Planted May 28)							
	July 18	*July 22*	*July 26*	*July 30*	*Aug. 2*	*Aug. 6*	*Ave.*	
Thimet 1[b]	230	2,870	3,290	6,070	9,530	15,030	6,170	
1[c]	160	2,590	3,660	5,230	9,320	14,650	5,935	
1[a]	180	2,450	3,420	5,553	8,990	14,160	5,792	
0.5[c]	720	3,520	3,940	6,290	11,810	15,770	7,008	
Toxaphene spray 0.75[a]	1,650	5,750	5,910	9,750	11,920	11,000	7,663	
Untreated check[a]	1,270	3,280	4,030	5,100	8,170	8,420	5,045	
	Experiment 3 (Planted May 28)							
Thimet 0.5[a]	3,320	6,120	6,300	7,930	—	13,390	7,412	
.5[c]	1,000	3,380	2,930	7,000	—	14,980	5,858	
Bayer 19639 0.6[a]	2,880	3,990	5,370	7,410	—	12,710	6,472	
1.2[a]	2,150	2,940	4,360	6,750	—	12,440	5,728	
Toxaphene spray 0.75[a]	8,020	8,870	5,760	8,180	—	8,220	7,810	
Untreated check[a]	4,224	3,986	3,750	5,100	—	5,810	4,574	

[a] Reginned seed.
[b] Flame-delinted seed.
[c] Acid-delinted seed.

were due to the earlier cotton fleahopper infestation. Again moisture conditions were such that plants were able to set a near-maximum crop despite the fruiting delay. The somewhat lower yields from toxaphene-sprayed plots or from plots planted with Thimet-treated-flame-delinted seed were due to land variation.

In experiment 3, in which noticeable phytotoxicity did not occur, cotton fleahopper and bollworm treatment was the same as in experiment 2. On June 18 there were fewer blooms in the treated-seed plots than in the check, and considerably fewer than in toxphene-sprayed plot, indicating a slight delay in fruiting due to seed treatment. On July 22 blooms in the Thimet-treated reginned-seed plot approached those in the toxaphene-sprayed plot, but the other seed treatments were on a par with the check. The lesser effectiveness against thrips and fleahoppers was probably responsible for this further delay in fruiting in these treatments. This delay was quite evident in the Thimet-treated acid-delinted-seed plot, as blooming continued for a week or more after it had ceased in the other plots. Moisture became depleted and plants failed to hold bolls set near the top, as plants in other plots which fruited earlier were able to do. Consequently yields were lower than in the other seed treatments. It gave a gain of 176 pounds of seed cotton per acre over the untreated check, but the other treatments gave gains of 373 to 455 pounds.

In experiment 4 records on delayed fruiting were not made, but observations indicated it was similar to that of experiment 1 located in the same field. Differences in yield between treatments were due to land variations.

REFERENCES CITED

Parencia, C. R., Jr., J. W. Davis, and C. B. Cowan, Jr. 1957a. Control of early-season cotton insects with systemic insecticides employed as seed treatments Jour. Econ. Ent. **50**(1): 31–9.

Parencia, C. R., Jr., J. W. Davis, and C. B. Cowan, Jr. 1957b. Further field tests with systemic insecticides employed as seed treatments. Jour. Econ. Ent. **50**(5): 614–7.

Field Studies with Systemic Insecticides against Cattle Grubs and Lice[1]

G. R. DeFoliart, M. W. Glenn, and T. R. Robb,[2] *University of Wyoming, Laramie*

Abstract

In tests involving 270 calves treated orally with single doses of systemic insecticides in bolus form, Dow ET-57 (Trolene) and Dowco-109 (phosphoramidothioic acid, methyl 1-, *O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ester), gave high kills of *Hypoderma lineatum* (DeVill.) and *H. bovis* (L.). Control with both insecticides exceeded 90%. Dow ET-57 appeared to be as effective at a dosage rate of 85 mg./kg. as at 110 mg./kg. Dimethoate (*O,O*,-dimethyl-S-(N-methylcarbamolymethyl) phosphorodithioate), also called Am. Cyanamid 12,880, gave only erratic control at rates of 15 and 10 mg./kg. None of the three insecticides, when given orally, killed 100% of cattle lice, but dimethoate produced higher kills than Dow ET-57 or Dowco-109. When given as feed additives to 160 calves, Dowco-109 at 2.0, 7.5, and 15 mg./kg. fed for 10, 2, and 1 day, respectively, and Dow ET-57 at 55 mg./kg. fed for 2 days, caused grub reductions of 61 to 82%. Better results might have been obtained under more normal feeding conditions than prevailed in this test. As a complete coverage spray, Bayer 21/199 (Co-ral) 0.25 and 0.5% (from wettable powder) provided excellent louse control for at least 5 months. Dowco-109 (wettable powder) provided good louse control for a period of from 3 to more than 4 months. Neither Bayer 21/199 nor Dowco-109 gave satisfactory control of grubs under the conditions of these spray tests, although each gave about 80% control in one test at a concentration of 0.5%. It was concluded that Bayer 21/199 might potentially provide "one-shot" grub and lie control on cattle.

McGregor & Bushland (1957) and Roth & Eddy (1957) reported the discovery of the effectiveness of Dow ET-57 (Trolene) as a systemic insecticide for control of cattle grubs (*Hypoderma lineatum* (DeVill.) and *H. bovis* (L.)). They found that Dow ET-57 killed all grub instars and grubs were therefore effectively controlled by a single oral treatment. Radeleff & Woodard (1957) reported on toxicological studies of Dow ET-57 in cattle and sheep.

Since the initial work with Dow ET-57, it and several other systemics and methods of administering them have been tested extensively under varying conditions and against a variety of external parasites throughout the United States and Canada. Results against grubs have been reported by Adkins (1957), Knapp *et al.* (1958), Marquardt & Fritts (1957), Raun & Herrick (1957 a, b), and others; results against cattle lice by DeFoliart & Glenn (1957); the nasal bot fly of sheep by Peterson *et al.* (1958); and demodectic mange in dogs by Gaafar & McDonald (1957) and Sanger (1958).

Given herein are the results of tests in Wyoming during the season of 1957–58 on cattle grubs and lice with systemic insecticides applied as single oral doses, as feed additives, or as dermal sprays.

Procedure.—In all tests except one, the test animals were calves born the previous spring. In general, these calves (short yearlings) and 2-year-olds are more heavily and more consistently infested than older animals. In the one exception mentioned above, 2-year-olds were used.

The data on grub reduction represent a single count of the species involved. An attempt was made to check the herds during the period when *H. lineatum* was present in the backs at nearly peak numbers and again when *H. bovis* was present in peak numbers.

Except for occasional early grubs, *H. lineatum* usually begins to arrive in the backs early in December in the areas where the test herds were located. However, grubs appeared later than usual during the winter of 1957–58. Examination of several herds between January 6 and January 8 resulted in the finding of only very small grubs or none at all in the backs of untreated cattle.

Bolus Treatments.—In five herds totaling 347 calves, 270 were treated orally with boluses. Each herd was divided into equal units so that the insecticides and dosage levels could be tested on a competitive basis. Calves were treated with Dow ET-57, Dowco-109, (phosphoramidothioic acid, methyl 1-, *O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ester) or dimethoate (*O,O*-dimethyl-S-(N-methylcarbamoylmethyl) phosphorodithioate), also called Am. Cyanamid 12, 880, with the treatments alternated as the calves came through the chute. Boluses were administered with a single-action balling gun. A total of 61 calves in 4 herds were treated with Dow ET-57 at 110 mg./kg., and 29 in 2 herds at 85 mg./kg.; 77 calves in 5 herds were treated with Dowco-109 at 15 mg./kg. and 13 in 1 herd at 10 mg./kg.; 69 calves in 4 herds were treated with dimethoate at 15 mg./kg. and 21 calves in 2 herds at 10 mg./kg. Herd No. 1 was treated on October 1, Herd 2 on October 31, Herd 3 on November 6, Herd 4 on November 27, and Herd 5 on December 18.

Feed Additives.—A test to determine the grub control that could be achieved by incorporating systemics at a high level in the feed for a short period was conducted in a herd of 200 calves. The insecticides used were Dow ET-57 and Dowco-109, each 10% on reprocessed wheatmill feed. They were mixed in a cement mixer with rolled barley containing 5% molasses on the basis of 40 350 lb. calves receiving 80 lbs. per day of the feed. The initial distribution of the wheatmill feed with the rolled barley was apparently good, but the evenness of the mixture cannot be known with certainty as the wheatmill feed did not adhere well to the molasses, and the difference in particle size may have resulted in some redistribution upon handling and transport.

The herd was divided into groups of 40 and a group fed on each of the following schedules:

Dowco-109, 2.0 mg./kg. daily for 10 days; Dowco-109, 7.5 mg./kg. daily for 2 days; Dowco-109, 15 mg./kg. for one day; Dow ET-57 55 mg./kg. daily for 2 days; and untreated calves receiving only the barley feed. Each of the groups was started on the feed on November 26, except the group fed for 10 days which was started on December 5.

[1] Published with approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 114. Accepted for publication July 14, 1958.

[2] Department of Entomology and Parasitology, Department of Veterinary Science and Bacteriology, and Extension Service, respectively, University of Wyoming, Laramie.

Dermal Spray Applications.—A total of 153 calves were sprayed with various concentrations of Bayer 21/199 (Co-ral) or Dowco-109 (as wettable powders) as shown in table 5. They were sprayed with a single-nozzle gun with a No. 6 orifice at pressures of 200 to 275 lbs. per sq. in. (except that the group in Herd No. 1 sprayed with 21/199 0.25% was sprayed at a pressure of only 125 lbs. per sq. in. because of pump trouble). Herd No. 1 consisted of coming 2-year-olds that were sprayed on November 7. In this herd, 2 gallons of spray material per animal were used as a complete coverage application. All other sprayed groups consisted of calves (short yearlings) and were sprayed between November 13 and December 18. By then, the hair was long and 1.2 to 1.7 gallons of spray material per calf were used in an effort to wet them thoroughly.

Grub counts were made on all animals in Herds 1 and 2. Louse counts were made on approximately one-half of the treated animals where the treated group consisted of 10 calves or less, and on approximately one-fourth of the animals in larger groups. Since the chance of spraying error is increased in larger groups, it is felt that the examination of a smaller percentage of the animals in such groups does not subject them to overly critical comparison with smaller treated groups.

Results.—*Bolus Treatments.*—Insecticides used and dosage levels are shown in table 1. Dow ET-57 at 110 or 85 mg./kg. and Dowco-109 at 15 or 10 mg./kg. both gave consistently high kills of both species of grubs. Each insecticide appeared equally effective against both species of grubs. There was no apparent difference in effectiveness of either insecticide at the two levels of administration. This is of particular interest in the case of Dow ET-57. Most range calves weigh about 350 to 400 lbs. by October and the above data indicate that calves of that size can be satisfactorily treated with one of the commercially available 15-gram boluses, thus saving the time and expense of using additional fractions of boluses for calves over 300 lbs. Dimethoate at 15 and 10 mg./kg. gave unsatisfactory control.

Post-treatment symptoms were seen in some calves in four of the five herds, but there seemed to be no definite pattern associated with feed or weaning status. The herd (No. 4) in which no adverse effects were noted consisted of weaned calves that were each receiving 1½ pounds of ground oats and ½ pound of pelleted alfalfa per day. Two weeks before treatment, many of the calves were suffering from shipping fever or pneumonia. In another weaned group (Herd No. 5), several of the calves became quite sick and went off feed for 1 day. Several were stiff-gaited. Dimethoate-treated calves were the most severely affected.

Only one unweaned group was treated. These calves were in Herd No. 2, and aside from their mothers' milk, their diet consisted only of native pasture. One calf treated with dimethoate and one treated with Dowco-109 walked rather "tender-footed"; one treated with dimethoate displayed obvious stiffness, and one treated with Dowco-109 was very weak in the hind quarters, being unable to run far without knuckling over at the pastern joints and falling.

Two herds (1 and 3) were treated the same day they were weaned. In Herd No. 1 (receiving crested wheat and alfalfa hay but no grain or protein supplement), six calves treated with Dowco-109 and three treated with dimethoate showed excessive salivation and one treated with dimethoate had a stiff, wobbly gait and appeared to be quite sick. In Herd No. 3 (receiving ground alfalfa hay and one-half pound of protein supplement per day), one calf treated with Dowco-109 and one treated with dimethoate salivated excessively, and one treated with Dowco-109 was stiff-gaited. The rancher stated later that these calves became droopy after we observed them and did not get fully back on feed for nearly 2 weeks.

Lice control studies with Dow ET-57 were made during 1956 (DeFoliart & Glenn 1957), and it was found that Dow ET-57 had little utility for louse control. Observations on lice control on a limited number of animals treated orally with Dowco-109 and dimethoate were made during 1957. In Herd No. 1, louse-infested areas of several square inches were marked on one calf treated with Dowco-109 and one calf treated with dimethoate. The lice present were *Solenopotes capillatus* Enderl. Three days after treatment, the number of lice in the marked area on the calf treated with Dowco-109 at 15 mg./kg. had been reduced from 39 to 18, a reduction of 51%; on the calf treated with dimethoate, at 10 mg./kg. lice in the marked area were reduced from 10 to 0, and no live lice could be found on the animal.

After this, two calves at the Veterinary Laboratory in Laramie were treated orally with dimethoate at 15 mg./kg., and two others with Dowco-109 at 15 mg./kg. to determine the effect on lice. *Linognathus vituli* (L.) was the species infesting these calves. Infested areas were

Table 1.—Control of cattle grubs with orally administered systemics (single doses).

Herd No.	No. Treated with each Insecticide	Average No. Grubs[a] Untreated Calves		Per Cent Reduction of Grubs						Per Cent of Cattle Infested							
				Dow ET-57[b]		Dowco-109[b]		Dimethoate[b]		Untreated		Dow ET-57		Dowco-109		Dimethoate	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	8	14.4	22.4	97	88	95	78	76[c]	41[c]	100	100	29	67	33	67	62	88
2	13	—	10.2	—	89	—	76	—	73	—	94	—	64	—	80	—	78
	13			—	92[d]	—	87[e]	—	58[c]			—	27	—	58	—	91
3	20	14.1	10.0	87	97	86	94	35	66	89	100	40	10	60	30	75	83
4	20	17.0	10.8	97	97	95	94	86	82	91	100	26	15	30	32	70	67
5	16	17.6	5.1	98[d]	98[d]	95	98	95	63	100	89	20	12	27	06	37	75

[a] A, denotes *H. lineatum;* B, denotes *H. bovis.*
[b] Dosage level: Dow ET-57, 110 mg./kg.; Dowco-109, 15 mg./kg.; dimethoate, 15 mg./kg.
[c] Dimethoate 10 mg./kg.
[d] Dow ET-57 85 mg./kg.
[e] Dowco-109 10 mg./kg.

marked as before. Forty-eight hours after treatment, lice in the marked area on one calf treated with dimethoate had been reduced from 11 to 2, a reduction of 82%, and on the other calf from 29 to 3, a reduction of 90%. Lice on the Dowco-109-treated calves were reduced from 18 to 7 and from 27 to 13, reductions of 61 and 52%, respectively.

In Herd No. 5, similar counts were made on calves treated with dimethoate at 15 mg./kg., but it was found that adult lice survived on three of five calves examined 5 days after treatment. These observations, though limited in number, indicate that single oral treatments of Dowco-109 or dimethoate will not give 100% mortality of cattle lice, and like Dow ET-57, they offer little in the way of practical louse control. Orally administered dimethoate, however, does appear to be definitely more toxic to cattle lice than either Dow ET-57 or Dowco-109.

Feed Additives.—With the exception of the group that was fed for 10 days, it was not possible to do the preparatory work such as ear-tagging and the actual feeding at separate times, so the handling involved and the fact that the calves were fed in strange bunkers made the conditions somewhat abnormal. Also, some calves were dehorned, but this did not seem to affect the appetites of those observed in the untreated group. Normally, a few calves will eat little or no feed or much less than their share on a given day, but probably this occurred to a greater extent than usual in this test, and grub reductions would have been higher under more normal conditions. The Dow ET-57-treated group found the feed unpalatable but did finally clean it up on both days.

Despite the adverse factors affecting this test, appreciable grub reductions were obtained (table 2). In each treated group, the fact that three or four calves carried a large number of grubs greatly reduced the amount of the reduction. These heavily infested calves probably consumed too little feed to adequately dose themselves. For example, in the group fed Dowco-109 at 2 mg./kg. for 10 days, three calves out of 34 that were examined had 58 of the 75 grubs of *H. lineatum* that were found. Later three other calves in this group accounted for 50 of 71 *H. bovis* found in 35 that were examined, and these three had also accounted for 10 *H. lineatum* in the earlier inspection.

In the group fed Dowco-109 at 7.5 mg./kg. for 2 days, four calves accounted for 90 *H. lineatum* of a total of 121 found in 38 calves. Later these same four calves had 36 of 96 *H. bovis* found in 36 calves that were examined. Two other calves carried 27 of the *H. bovis* total. The other treated groups had a similar pattern.

Lice control was evaluated in the group fed Dowco-109 for 10 days. The infestation consisted of *S. capillatus* and *L. vituli* and some animals were heavily infested. Eight calves were examined at the end of the feeding period. Some dead lice were found in the hair and some patches of *S. capillatus* had a "thinned-out" appearance, but each of the eight calves still had lice and four were still heavily infested, two animals having *capillatus* numbering 35 and 45 per square inch in the heavier areas.

The calves of all groups except the group fed for 10 days were observed at 4:00 P.M. on November 27 for symptoms of toxicity. Impending darkness prevented anything more than a general survey of the situation. About 15 to 20% of all three groups displayed decided stiffness and another 15 to 20% slight to moderate stiffness. Many of these were droopy and obviously not feeling well. Excessive salivation was noted in only two or three calves. The group fed 15 mg./kg. of Dowco-109 in one feeding showed more pronounced symptoms of toxicity than the other treated groups. These apparently had not gone to water for nearly 24 hours after treatment. Two of them were bloated. By the morning of November 28, all calves appeared completely normal.

Minor symptoms were also observed by the owner in the group fed Dowco-109 at 2 mg./kg. for 10 days. He reported that one calf was quite stiff and several others slightly so and three calves were somewhat bloated on the day following the first feeding. No symptoms were observed after that. These observations of the rancher are considered quite reliable as he had seen quite a few affected animals during the course of our tests in his herd.

Under Wyoming conditions, administering chemicals as feed additives for grub control ordinarily would be a special operation since few herds receive supplementary feed early in the fall when they should be treated. For this reason, keeping the feeding period short is important. This test indicates that under normal feeding conditions, short-term feeding can be successfully accomplished.

Spray Applications.—In most of these tests the main objective was to determine louse control. Herds 1 and 2 were divided into lots kept separate from each other so that untreated animals were available for grub counts. All of the calves in Herds 3 to 5 were sprayed.

Neither Bayer 21/199 nor Dowco-109 gave outstanding grub control in these tests (table 3). When Herd No. 1 was examined in January, 28 untreated calves averaged 4.4 *H. lineatum* per animal. In February these same calves averaged 16.2 *H. lineatum*. The data show that both Bayer 21/199 and Dowco-109 gave about 80% grub re-

Table 2.—Control of cattle grubs with high-level systemics in feed.

Insecticide Level (Mg./Kg.)[a]	Feeding Period (Days)	Average No. of Grubs[b] per Calf		Infestation Range		Per Cent Reduction		Per Cent Infested	
		A	B	A	B	A	B	A	B
Untreated	—	12.1	9.8	0–66	1–24	—	—	73	100
Dowco-109, 15	1	2.8	3.0	0–38	0–20	77	69	41	49
Dowco-109, 7.5	2	3.2	2.7	0–42	0–15	74	72	39	47
Dowco-109, 2.0	10	2.2	2.0	0–34	0–21	82	80	26	26
Dow ET-57, 55	2	4.7	2.2	0–45	0–14	61	78	60	58

[a] Data based on 40 calves in each instance.
[b] A, denotes *H. lineatum;* B, denotes *H. bovis.*

Table 3.-Cattle grub and lice control with systemics applied as dermal sprays.

Herd No.	Insecticide Concentration (Per Cent)	No. Treated	Date Sprayed	Spray Pressure (Lbs./ Sq. In.)	Amount Applied per Calf (Gals.)	Per Cent Grub Reduction		No. of Calves Infested with Lice[a]		
						Jan.	Feb.	1 mo.	3 mo.	4–5 mo.
1	Bayer 21/199, 0.25	7	Nov. 7	125	2	39	07	0 (3)	0 (3)	0 (4)
	Bayer 21/199, 0.5	7	Nov. 7	275	2	86	75	0 (3)	0 (3)	0 (4)
	Dowco-109, 0.25	7	Nov. 7	275	2	45	49	0 (3)	0 (3)	1 (4)
	Dowco-109, 0.5	7	Nov. 7	275	2	80	80	0 (3)	1 (3)	0 (4)
2	Dowco-109, 0.75	10	Nov. 13	250	1.5	51	—	1 (5)	2 (5)	—
3	Bayer 21/199, 0.25	41	Dec. 11	200	1.2	—	—	0 (10)	0 (5)	2 (10)
4	Bayer 21/199, 0.5	44	Dec. 18	200	1.7	—	—	0 (10)	1 (10)	3 (10)
5	Dowco-109, 0.25	30	Dec. 12	250	1.7	—	—	1 (7)	—	—

[a] The number of calves examined is shown in parenthesis.

duction at a concentration of 0.5%. Poor control was achieved with both insecticides at a concentration of 0.25%. There is no readily apparent reason for the poor control achieved with Dowco-109 at 0.75% in Herd No. 2.

Untreated controls were not available in Herds 3 and 4, but good control was not achieved as evidenced by the fact that in January Herd No. 3 averaged 5.7 grubs per calf and Herd No. 4 averaged 12.5 grubs per calf.

Bayer 21/199 gave outstanding louse control. No lice were found on calves in Herd No. 1 sprayed with 0.25 or 0.5% through the observation period of 111 days, which was terminated by slaughter of the animals. In Herd No. 3, sprayed with Bayer 21/199 0.25% four lice (*L. vituli*) were found on two of 10 calves examined 139 days after spraying. In Herd No. 4, sprayed with Bayer 21/199 0.5%, five lice (*L. vituli*) were found on three of 10 calves examined 131 days after spraying. Both of these herds were heavily infested with lice before treatment.

Dowco-109 also appeared promising for cattle-lice control, although in this particular set of tests it did not quite match that obtained with Bayer 21/199. No toxic symptoms were noticed in any sprayed cattle.

It is concluded from these tests that Bayer 21/199 or other insecticides of similar action potentially offer "one-shot" grub and lice control, but that much remains to be learned about application of the insecticide to obtain effective grub control. In these tests the animals were wetted to the point of run-off, and the degree of wetting to the skin was at least equivalent to, if not more than, what the average stockman will achieve with standard spraying equipment and practice.

References Cited

Adkins, T. R., Jr. 1957. Field evaluations of Dow ET-57 as a systemic insecticide for the control of the common cattle grub in Alabama. Jour. Econ. Ent. **50**(4): 474–6.

DeFoliart, G. R., and M. W. Glenn. 1957. Cattle grub and lice control with Dow ET-57 in Wyoming. Down to Earth. **14**(3): 4–5.

Gaafar, S. M., and C. N. McDonald. 1957. A preliminary report on the treatment of demodectic mange in dogs. The Southwestern Veterinarian **10**(4): 309–10.

Knapp, F. W., C. J. Terhaar, and C. C. Roan. 1958. Field studies with feed and bolus formulations of Dow ET-57 for control of cattle grubs. Jour. Econ. Ent. **51**(2): 119–22.

Marquardt, W. C., and D. H. Fritts. 1957. Control of cattle grubs by an orally administered organic phosphorus compound. Jour. Amer. Vet. Med. Assoc. **131**(12): 562–3.

McGregor, W. S., and R. C. Bushland. 1957. Tests with Dow ET-57 against two species of cattle grubs. Jour. Econ. Ent. **50**(3): 246–9.

Peterson, H. O., E. M. Jones, and N. G. Cobbett. 1958. Effectiveness of Dow ET-57 (Trolene) against the nasal botfly of sheep. Amer. Jour. Vet. Res. **19**(70): 129–31.

Radeleff, R. D., and G. T. Woodard. 1957. Toxicological studies of Dow ET-57 in cattle and sheep. Jour. Econ. Ent. **50**(3): 249–51.

Raun, E. S., and J. B. Herrick. 1957. Feedlot tests of the efficacy of Dow ET-57 (Trolene) for control of cattle grubs. Jour. Amer. Vet. Med. Assoc. **131**(9): 421–3.

Raun, E. S., and J. B. Herrick, 1957. Clinical test of the efficacy of Dow ET-57 for grub-control in cattle. Jour. Econ Ent. **50**(6): 832.

Roth, A. R., and G. W. Eddy. 1957. Tests with Dow ET-57 against cattle grubs in Oregon. Jour. Econ. Ent. **50**(3): 244–6.

Sanger, V. L. 1958. ET-57 (Trolene) for demodectic mange —a case report. Jour. Amer. Vet. Med. Assoc. **132**(2): 56–7.

A Comparative Study on the Speed of Action of Some Halogen-Containing Thiol Alkylating Agents on Resistant House Flies[1]

S. Bettini, M. Boccacci, and G. Natalizi,[2] *Department of Parasitology, Istituto Superiore di Sanità, Rome, Italy*

Abstract

A comparative study was made on the speed of action of iodo- bromo- and chloroacetic acids and myristyl and cetyl bromo- and chloroacetates on *Musca domestica*. The usual contact methods were employed and a strain (K_1) highly resistant to DDT was used.

The speed of action was shown to be much higher for iodo- and bromoacetic acids and for the bromoacetates than for chloroacetic acid and the chloroacetates.

The differences in speed of action are compared with the speed of reaction between the free acids and cysteine (Barron) where a striking difference between iodo- and bromoacetic acid on one side and chloroacetic acid on the other, can also be noted.

The authors infer that the differences in speed of action between the halogen-containing thiol alkylating substances under experiment should depend upon their different chemical reactivity toward thiol-containing substances.

In a previous paper on the toxic properties of chloroacetic acid and of some of its esters (halogen-containing thiol alkylating agents), on resistant house flies (Boccacci & Bettini 1956), mention was made of the LD_{50} values of iodo-, bromo- and chloroacetic acid for a strain of *Musca domestica* L. (R-Sardinia), highly resistant to chlordane and appreciably resistant to DDT and its analogues and to lindane. The LD_{50} values were respectively, 1.5, 1.5, 2.5 gm./m.^{2}.

In a following paper Ascher (1957), using a continuous tarsal contact method, observed that the knockdown activity of bromo-, iodo- and chloroacetic acids, toward two strains of *M. domestica* (a susceptible and a resistant one), decreased in that order. In the same paper the author pointed out that a series of bromoacetates had shown a knockdown activity much higher than for the corresponding chloroacetates.

The present work aims to compare the toxicities of the three halogenoacetic acids to *M. domestica*, and in particular their speed of action. The research has also been extended to myristyl and cetyl bromo- and chloroacetates, products with low volatility, as representing the esters of the above mentioned acids.

Biological Material.—The experiments have been carried out on a strain of *M. domestica* (K_1)[3] highly DDT-resistant, and kept under constant selection against DDT.

K_1-strain has been bred in our laboratory following the usual methods: the larvae have been fed wheat-bran 1 part, water 2 parts and milk 0.025 parts by weight; aluminum containers with about 600 to 700 grams of breeding medium have been used. From each container about 1000 pupae of relatively uniform size have been obtained. For toxicity tests, 3-day-old females, average weight about 20 mg. have been employed. Experiments have been carried out at room temperature (24 to 26° C.).

Products Employed.—Iodo-, bromo- and chloroacetic acids: pure products for analysis (m.p.: 81–83°, 48–50°, 60–63°, respectively).

Myristyl bromoacetate (b.p. 157–160°/0.4 mm.), myristyl chloroacetate (b.p. 150–153°/0.5 mm.), cetyl bromoacetate (b.p. 184–186°/0.4 mm.) and cetyl chloroacetate (b.p. 159–163°/0.2 mm.), synthesized by one of us (Boccacci).

Methods.—In studying the speed of action of iodo-, bromo- and chloroacetic acid, highly volatile substances, a method already described (Bettini & Boccacci 1955) has been employed. Such a method consists in the use of filter paper cylinders treated with the substances dissolved in olive oil-ether 1:2. The time of contact has been 1 hour.

For the esters, substances having low volatility, the following method has been used. Petri dishes (covers only) of 10 cm. in diameter were treated with known concentrations of the esters in acetone solutions. At 24 hours after treatment, groups of 10 female flies under slight anesthesia were placed into each dish. The petri dishes were then covered with squares of cotton net[4] which were fastened by means of brass rings with slightly conic sections. Ten petri dishes (100 flies) were used for each concentration. The flies were kept in the dishes 30 minutes and throughout this period the net was slightly stricken with a plastic fly-swab so as to drive away the insects resting on the net and to force them to walk on the treated surface. After the 30 minutes the flies, anesthetized with CO_2, were transferred, in groups of 20 individuals each, to 250-cc. glass jars where small tubes containing water and closed with cotton plugs, were placed. The jar corks bore a hole (1 cm. in diameter) covered by a net. Since the knockdown of these substances is irreversible, in calculating the LD_{50} values, all knocked down individuals were counted. Knockdown readings were limited to 24 hours since the differences between the LD_{50} values at 24 hours and at 48 hours were very small, as shown in table 1.

The method thus described gives constant and reproducible results.

Results.—As can be seen from figure 1, iodo-, bromo- and chloroacetic acids show a speed of action decreasing

Table 1.—LD_{50} (gm./m.^{2}) at 24 and 48 hours for myristyl bromoacetate and myristyl chloroacetate. *Musca domestica* (K_1-strain).

Myristyl Bromoacetate		Myristyl Chloroacetate	
24 Hrs.	48 Hrs.	24 Hrs.	48 Hrs.
0.22	0.21	0.43	0.42

[1] Partial cost of publication of this paper was met by the Istituto Superiore di Sanità, Rome, Italy. Accepted for publication July 30, 1958.

[2] The authors wish to thank Mr. G. Pierdominici for helpful assistance.

[3] The K_1-strain has been delivered to us by Dr. C. Kocher of the J. R. Geigy Co., Basle, through the kind attention of Capt. K. R. S. Ascher, both of whom we sincerely thank.

[4] The net is used to decrease the toxic effects due to the vapours.

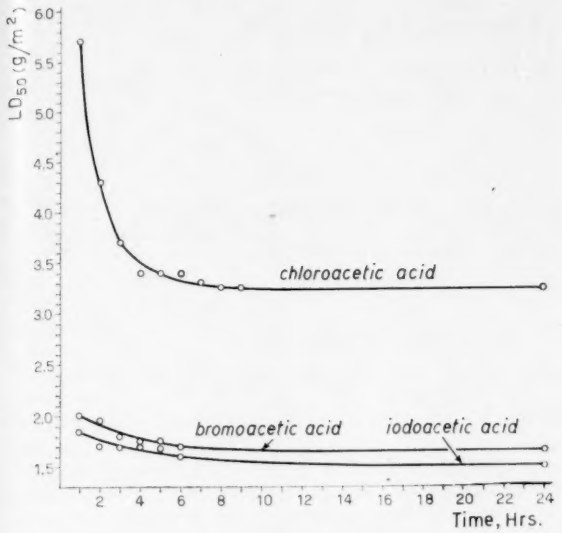


FIG. 1.—Comparative speed of action (k.d.) of iodo-, bromo- and chloroacetic acids on female house flies.

in order. The knockdown at different times for the various concentrations of iodo- and bromoacetic acids tends to reach, within the first 2 hours after contact, very high values which vary little during the following 22 hours. In the case of chloroacetic acid, however, the phenomenon was a slower one, and 8 to 9 hours were needed for the knockdown figures to reach the highest values. The LD_{50} values at 24 hours were about the same for iodo- and bromoacetic acids, 1.50 gm./m.² and 1.65 gm./m.², respectively; while the LD_{50} for chloroacetic acid was about two times higher, 3.25 gm./m.².

An analogous phenomenon can be observed for myristyl and cetyl bromoacetates, and myristyl and cetyl chloroacetates. In this case also, as can be seen from figures 2a and 2b, the speed of action is much faster for the bromoacetates (about highest knockdown within 5 to 6 hrs.), than for the chloroacetates (8 to 9 hrs.). The bromoacetates and chloroacetates show, moreover, the same ratio (about 1:2) between their LD_{50} values at 24

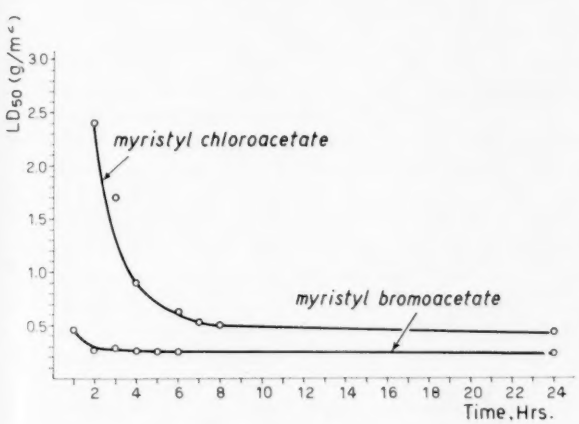


FIG. 2a.—Comparative speed of action (k.d.) of myristyl bromoacetate and myristyl chloroacetate on female house flies.

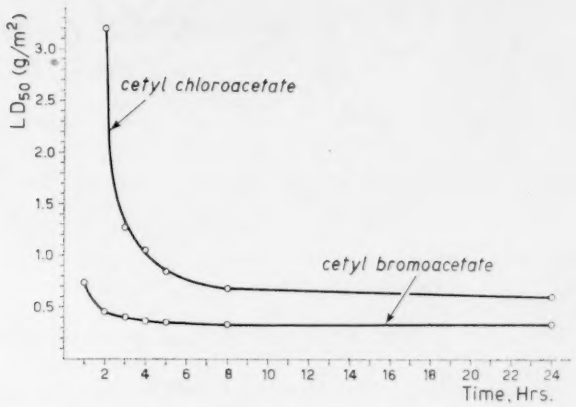


FIG. 2b.—Comparative speed of action (k.d.) of cetyl bromoacetate and cetyl chloroacetate on female house flies.

hours as has been observed for bromo- and chloroacetic acid (myristyl bromoacetate 0.23 gm./m.², myristyl chloroacetate 0.44 gm./m.²; cetyl bromoacetate 0.33 gm./m.², cetyl chloroacetate 0.62 gm./m.²).

DISCUSSION AND CONCLUSIONS.—It must first be noted that the present results confirm the ones reported in a preceding paper (Boccacci & Bettini 1956), *i.e.*, the ratio between the LD_{50} values of iodo- and bromoacetic acids on one side, and of chloroacetic acid, on the other, is about 1:2.

However, there is a marked difference on speed of action between iodo- and bromoacetic acid, myristyl and cetyl bromoacetates, on one side, and chloroacetic acid, myristyl and cetyl chloroacetates, on the other, especially so during the first few hours after contact.

If one compares the speed of action of these substances (LD_{50} values against time)[5] (figures 1 and 2) with the *in vitro* reactivity of the halogenoacetic acids with cysteine (Barron 1951) (fig. 3), a close analogy between the two cannot be overlooked. In fact, in Barron's experiment the speed of the reaction is strikingly higher for iodo- and bromoacetic acids than for chloroacetic acid. As a parallel, in our experiments the speed of action of iodo- and bromoacetic acids and of the bromoacetates is higher than the one of chloroacetic acid, and of the chloroacetates.

The analogy between chemical and biological phenomena is more evident by comparing the graphs reported by Barron with those obtained by plotting the reciprocals of the LD_{50} values on the ordinate and time on the abscissa (fig. 4).

If can be inferred, therefore, that the differences in speed of action between the halogen-containing thiol alkylating substances under experiment should depend on their different chemical reactivity toward thiol-containing substances.

The data of the present work contrast only in appearance with those reported by Ascher (1957). In fact, the marked differences in toxic action noted by him between bromo- and chloroacetates are due to the fact that the

[5] According to the graphic method of representing three variants (dose, knockdown and time) as employed by Beard (1949) and by McIntosh (1951) for comparing substances with different speed of action (or various formulations and/or environmental conditions which might affect the speed of action).

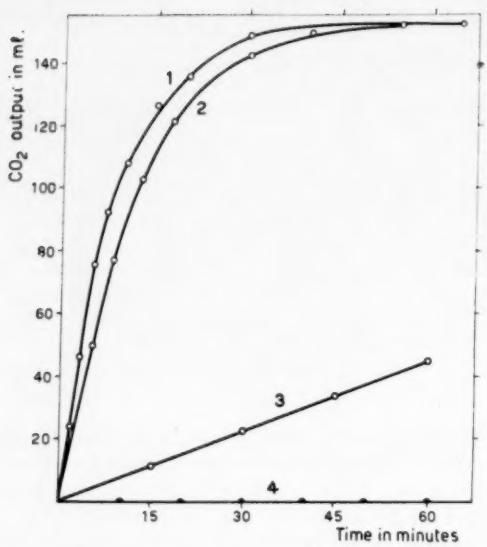


FIG. 3.—Combination of halogen acids with cysteine. Ringer bicarbonate buffer, gas phase, N_2:CO_2, pH 7.4, 28° C. Cysteine 300 μM in 3 ml.; halogen acid 70 μM in 0.5 ml. Abscissa, time in minutes. Ordinate, CO_2 output in cubic millimeters. (1) Iodoacetate. (2) Bromoacetate. (3) Chloroacetate. (4) Fluoroacetate. (from Barron 1951).

knockdown readings were limited to a period of 2 hours. In his experiments, he employed a method by which the insects were kept in continuous contact with the substance under test, and the knockdown was recorded at fixed times, within a period of 2 hours only.

As it clearly appears from our data on the esters the differences between the LD_{50} values of the bromo- and the chloroacetates, are marked within the first 2 hours (ratio 1:7 to 1:9) and become smaller as time elapses until they reach after 24 hours a ratio of 1:2.

Ascher found that the toxicity (knockdown) differences between iodo- and bromoacetic acids, on one side, and chloroacetic acid, on the other, were less evident than those found between bromoacetates and chloroacetates. This can be explained by the lower differences in speed of action between the free acids: the ratios between LD_{50} values of iodo- and bromoacetic acid, on one side, and chloroacetic acid, on the other, are 1:2 at 24 hours and only 1:3 at 3 hours. For the discussion on the importance of the time factor in comparing the toxicity of different insecticides, the reader is referred to the work by Beard (1949).

Ascher's findings *i.e.*, bromoacetic acid more active

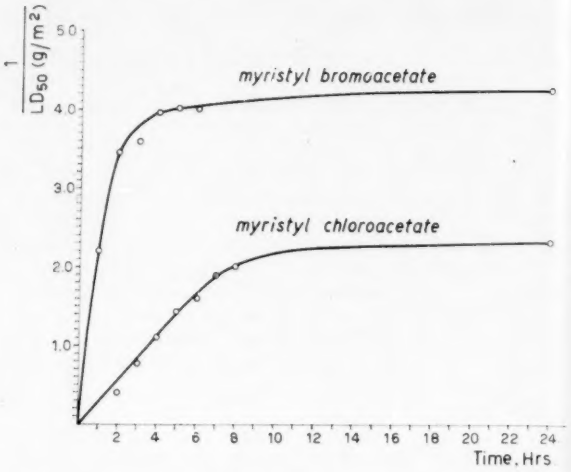


FIG. 4.—Reciprocals of LD_{50} values for female house flies in contact with myristyl bromoacetate and myristyl chloroacetate, against time.

than iodoacetic acid, could be explained by the fact that he employed very high doses (6 to 10 gm./m.²) compared with the ones used in our work (1 to 4 gm./m.²). The present research clearly demonstrates that in order to evaluate toxicity differences, even between substances having a similar chemical nature and the same mode of action, it is important to depend upon data which give a clear picture of knockdown against time, since there might be very marked differences in speed of action between the substances under test.

REFERENCES CITED

Ascher, K. R. S. 1957. Enhanced susceptibility of a DDT-resistant strain of house flies towards alkyl and aryl bromoacetates. Riv. Parassitol. **18:** 185–97.

Barron, E. S. G. 1951. Thiol groups of biological importance. Advances enzymol. **11:** 201–66.

Beard, R. L. 1949. Time of evaluation and the dosage response curve. Jour. Econ. Ent. **42:** 579–85.

Bettini, S., and Boccacci, M. 1955. Azione tossica degli acidi iode-e cloroacetico sugli insetti. Inibizione della triosofosfato deidrogenasi. Riv. Parassitol. **16:** 13–29.

Boccacci, M., and Bettini, S. 1956. Potere insetticida per contatto dell'acido cloroacetico e di alcuni suoi esteri per le mosche "resistenti." Rend. Inst. Super. Sanità **19:** 1237–46.

McIntosh, A. H. 1951. Particle size of insecticidal suspensions and their contact toxicity. III. Temperature coefficients and tests by injection. Ann. Appl. Biol. **38:** 567–76.

Chemical Control of *Hylemya antiqua* (Meig.) in the Pacific Northwest[1]

ANGUS J. HOWITT[2]

ABSTRACT

Strains of onion maggot (*Hylemya antiqua* (Meig.)) highly resistant to the chlorinated hydrocarbon insecticides appeared in certain areas of the Pacific Northwest. Furrow treatments, seed treatments, and multiple surface applications of chlorinated hydrocarbons were ineffective. Seed, soil and surface treatments with organic phosphates did not give economic control. Furrow treatments of Guthion, Diazinon, ethion, Trithion, V-C 13, and Bayer 21/199 gave excellent control. Furrow treatments with malathion, Dipterex, Perthane, Bayer 19639, Sevin, Thiodan, DDT, Kepone, Dow ET-57, and endrin were ineffective. Thimet gave good control as a furrow treatment, but was phytotoxic. Parathion applied in the furrow protected onions for several weeks, then declined rapidly.

The onion maggot, *Hylemya antiqua* (Meig.), is the most serious pest of onions in Washington. The pest is present everywhere in the State where onions are commercially grown. During the years 1954–57 this insect has caused severe damage in the onion fields throughout Washington. Prior to 1953 the infestations were light and readily controlled with aldrin, heptachlor, chlordane, and dieldrin as these became available. These materials were used as furrow, seed, and surface treatments. After 1953 the chlorinated hydrocarbons became less effective. In cooperative experiments conducted in Washington, British Columbia, Oregon, and Idaho; Finlayson *et al.* (1958) demonstrated conclusively that strains of onion maggot highly resistant to the chlorinated hydrocarbons were present in certain areas of the Pacific Northwest. This paper deals with tests conducted in Western Washington from 1953, when the chlorinated hydrocarbons began to fail, to 1958 when successful control measures were developed using organic phosphates.

Methods and Materials.—Five replications of single rows in a randomized block design were employed in all experiments. Each replication consisted of 10 lineal feet of row, except for experiments carried out in 1957 and 1958 for which 20 lineal feet per replication were employed. Dosages are stated in pounds of actual ingredient per acre or in ounces of actual ingredient per 1 pound of seed for seed treatments. When furrow treatments were employed, dosages were calculated on the basis of the number of lineal feet in an acre with rows 15 inches apart. In tables 2, 3, 4, 6, 7, 8, 9, 10, and 11, Duncan's Multiple Range Test was employed for statistical analysis of the data at the 5% level. Where this method is used, if two treatments do not have a common letter for a given column, then the treatments are significantly different (see table 3).

The midseason maggot-damage appraisals were made by frequent examination of the plots. Wilted plants were inspected to confirm onion maggot damage. When maggots were found in a plant, a slender wooden applicator, 3 inches in length, was placed along side the infested plant. Using this method, the data could be analyzed for any period during the course of the experiment. Since most damage was in the seedling stage, and the infested seedling onions wilted and disappeared, this means of recording damage proved invaluable.

Tests in 1953.—The chlorinated hydrocarbons were applied as surface sprays or furrow treatments. For furrow treatments a measured amount of dust or granulated material was placed in the furrow after seeding. The material was evenly distributed with a plastic spoon. The seed was planted with a Planet Jr. seeder with the shoe removed so that the furrow remained open after seeding. After the toxicant was placed in the furrow, the shoe was replaced and the furrow closed with the same seeder. The surface applications were sprayed in a 1-foot band over the rows using a 3-gallon hand sprayer. When a single surface spray was employed, it was applied at the crookneck stage. When two surface sprays were used, one-half the dosage was applied at the crookneck stage and the remainder 3 weeks later.

In the first field test, evaluation of toxicants consisted of selecting 200 plants at random from each treatment at harvest and examining them for damage. Later when it was observed that many plants were killed early in the season, it was obvious that per cent infestation, based on a random sample at harvest, was not a true indication of the effectiveness of a treatment. The figures on injury, given in table 1, are therefore conservative, but still serve to indicate the ineffectiveness of the chlorinated hydrocarbons as surface sprays.

Tests in 1954.—These tests consisted of screening chlorinated hydrocarbons using furrow, seed, and surface treatments and combinations of these methods. The furrow and surface treatments were applied as in the 1953 tests. When three surface sprays were used the last spray was applied 6 weeks after the crookneck stage. For the seed treatments, the correct amount of seed for each replication was obtained by first weighing the seed, then the samples were placed individually in glazed evaporating dishes and moistened with 3 or 4 drops of water. Uniform distribution of the insecticide was obtained by placing the wettable powder in a small glass vial. The powder was added to the seed by gently tapping the vial. The seed and insecticide were stirred with a glass rod after each portion of the chemical was applied. When the chemically coated seeds had dried, they were poured into a glass vial and stoppered with absorbent cotton. Uniformity of seeding was achieved by weighing an equivalent amount of seed for each 10-foot row.

Tests in 1956.—In 1956 the organic phosphates were

[1] Scientific Paper No. 1762, Washington Agricultural Experiment Station, Pullman. Project No. 1346. These investigations were partially supported by research grants from Velsicol Chemical Corporation, Shell Chemical Corporation, American Cyanamid Company, Stauffer Chemical Company, and Chemagro Corporation. Accepted for publication August 11, 1958. Partial cost of publication of this paper was met by the Department of Entomology, Washington Agricultural Experiment Station, Pullman.

[2] Associate Entomologist, Western Washington Experiment Station, Puyallup. Grateful acknowledgment is made to Lee Bennett and Stanley Cole for their assistance in carrying out these studies. The author wishes to thank the E. S. Gandrud Company, Incorporated, for the donation of equipment.

Table 1.—Percentage infestation and number of sound onions harvested from surface and furrow treatments. Puyallup, Washington, 1953.

Insecticide	Pounds per Acre	Per Cent Infestation
Two surface E.C. treatments. Last treatment to harvest 105 days		
Untreated		47.5
Chlordane	0.5	33.0
Isodrin	0.5	30.0
Chlordane	1.0	28.5
Isodrin	1.0	24.5
Heptachlor	0.5	23.5
Aldrin	1.0	19.5
Aldrin	0.5	18.0
Heptachlor	1.0	15.5
		Total No. Sound Onions
Last treatment to harvest 99 days		
Two surface E.C. treatments		
Chlordane	2.0	385
Aldrin	2.0	416
Heptachlor	2.0	435
Endrin	0.5	531
One surface E.C. treatment		
Chlordane	0.5	287
Chlordane	1.0	374
Heptachlor	1.0	403
Aldrin	2.0	422
Endrin	0.5	428
Heptachlor	2.0	459
Aldrin	1.0	479
Untreated		179
Furrow treatment. Treatment to harvest 139 days		
Untreated		49
Chlordane dust	2.5	226
Aldrin dust	1.0	249
Chlordane granules	2.5	314
Heptachlor dust	1.0	484
Dieldrin dust	0.5	493
Heptachlor granules	1.0	575
Isodrin dust	0.5	623

screened as seed, surface, and soil treatments. Seed and surface treatments were applied in the manner prescribed previously. For the soil treatments, in order to conserve space, but still simulate total soil treatment methods, the correct amount of toxicant for each 10-foot row was mixed with 2 quarts of water and sprinkled from a watering can in a 2-foot band. Immediately afterward, the treated area was "rototilled" into the soil 5 or 6 inches deep using a 24-inch Rototiller. The rows were smoothed and packed with a heavy roller, then seeded down the middle with a rod-row multiple gear seeder. The multiple gear seeder is designed to deliver to the ground a previously weighed or measured quantity of seed and insecticide in a predetermined length of drill row at an even depth and uniform spacing.

Tests in 1957.—All insecticides tested in 1957 were applied as furrow treatments. A Gandy chemical attachment mounted on a motor driven Planet Jr. seeder was used to apply the granules. By using this equipment it was possible to place the seed and toxicant in the furrow simultaneously. When dusts or wettable powders were used, a multiple gear seeder was used to place chemical and seed in the furrow at the same time.

Tests in 1958.—The methods employed in 1958 were similar to those of 1957 in that all insecticides were applied as furrow treatments. In the DDT-ethion treatments, equal weights of each toxicant were mixed together, then added to the Gandy granulated chemical attachment. Since both formulations consisted of 5% on 30/60 AA RVM attaclay, it can be assumed that the rate listed for this mixture consisted of equal amounts of both toxicants. The work in 1958 consisted of a single experiment involving the most promising compounds used at 1, 2, and 3 pounds actual per acre. A simple split-plot design using three checks was employed. Each check consisted of five replications.

Results.—See tables 1 to 11. Tables 1 and 5 give summaries of three and two individual experiments respectively. Since the work summarized in each table was conducted at the same time in the same field under similar conditions, it is presented in one table to conserve space. Since a total of 45 treatments were involved in table 11, three checks, each consisting of five replications, were included. The results are listed separately to show the severity of the infestation.

Discussion.—A study of tables 1, 2, and 3 indicates that the chlorinated hydrocarbons gave better results in 1953 and 1954 employed as furrow treatments than when they were used as post-emergence surface sprays. These toxicants appear to deteriorate far more rapidly on the surface of the ground, especially in bright warm weather,

Table 2.—Number of sound onions harvested from seed, furrow and surface treatments. Puyallup, Washington, 1954.[a]

Insecticide	Pounds per Acre	Total No. Sound Onions	Analysis
Seed treatments[b]			
Chlordane		141	a
Heptachlor		500	fgh
Seed treatment plus one surface treatment			
Chlordane	2.5	139	a
Heptachlor	1.0	503	fgh
One surface treatment			
Dieldrin	1.0	292	d
Endrin	1.0	391	e
Isodrin	1.0	453	ef
Chlordane	5.0	531	ghij
Heptachlor	2.5	606	kln
Aldrin	2.5	648	nop
Two surface treatments			
Dieldrin	1.0	405	e
Isodrin	1.0	481	fg
Endrin	1.0	508	fgh
Chlordane	5.0	582	jkln
Aldrin	2.5	609	klno
Heptachlor	2.5	674	op
Furrow treatment			
Aldrin	1.0	228	bc
Chlordane	3.5	268	cd
Heptachlor	1.5	557	hijk
Dieldrin	1.0	624	lnop
Isodrin	0.5	687	p
Furrow treatment plus one surface treatment			
Chlordane	6.0	236	bcd
Aldrin	3.0	251	cd
Heptachlor	2.5	513	fghi
Isodrin	1.0	576	ijkl
Dieldrin	1.5	591	jkln
Untreated		182	ab

[a] Onions planted May 1, 1954; harvested September 11 1954
[b] All seed treatments received 1 ounce of actual toxicant per 1 pound of seed.

Table 3.—Plant emergence and number of sound onions harvested from furrow and surface treatments. Puyallup, Washington, 1954.[a]

Insecticide	Pounds per Acre	Plant Emergence: Total No.	Plant Emergence: Analysis[b]	Non-Infested Onions: Total No.	Non-Infested Onions: Analysis[b]
One surface E.C. treatment					
Aldrin	2.5	519	c	365	d
Heptachlor	2.5	503	c	373	de
Two surface E.C. treatments					
Heptachlor	5.0	475	abc	204	b
Aldrin	2.5	346	ab	223	b
Aldrin	5.0	436	c	297	c
Heptachlor	2.5	492	bc	358	d
Three surface E.C. treatments					
Aldrin	3.5	488	bc	299	c
Heptachlor	3.5	502	c	374	de
Furrow dust treatment					
Isodrin	1.5	482	bc	409	ef
Isodrin	0.5	516	c	452	f
Dieldrin	2.0	492	bc	314	c
Untreated		512	c	10	a

[a] Onions planted July 15, and harvested October 7, 1954.
[b] Duncan's Multiple Range Test—if two treatments do not have a common letter for a given column, then the treatments are significantly different at the 5% level. Conversely, if two treatments in a given column share a common letter then these treatments are not significantly different.

than when they are covered with a protective covering of soil.

Two or three surface sprays generally did not produce better results than single applications. Onion growers in the area obtained a good measure of control by applying surface sprays of malathion every 5 days or DDT at 10-day intervals. Using this spray schedule, the residual actions of successive sprays overlapped so that the plants were always protected. However, even with this accelerated spray program, some damage occurred because the gravid females were able to deposit eggs before they expired. There were no peak population periods. The flies were present in the field in large numbers from early April until October, so that it was not possible to control the flies by one or two surface sprays applied to coincide with emerging broods.

Chlordane was least effective whether used as a seed,

Table 4.—The number of sound onions at midseason and at harvest-time using soil and seed treatments. Puyallup, Washington, 1956.[a]

Insecticide	Application: Method	Application: Pounds per Acre[b]	Sound Onions at Midseason: Total No.	Sound Onions at Midseason: Analysis	Sound Onions at Harvest: Total No.	Sound Onions at Harvest: Analysis
Thiodan E.C.	soil	5 lb.	216	a	26	a
Untreated			101	a	40	ab
Hercules AC-528 (E.C.)	soil	5 lb.	522	cd	68	ab
Endrin E.C.	soil	5 lb.	314	a	83	b
Endrin W.P.	seed	3 oz.	464	bc	232	c
Endrin W.P.	seed	4.5 oz.	459	b	247	c
Parathion E.C.	soil	5 lb.	549	de	336	d
Trithion W.P.	soil	5 lb.	617	f	379	d
Endrin W.P.	seed	6 oz.	607	ef	388	d
Ethion E.C.	soil	5 lb.	718	g	603	e
Thimet E.C.	soil	5 lb.	790	h	681	e
Diazinon E.C.	soil	5 lb.	826	gh	756	e

[a] Onions planted July 12, 1956 and harvested September 18, 1956.
[b] Seed treatments are expressed in ounces of actual ingredient per pound of seed. Soil treatments are expressed in pounds of actual ingredient per acre.

Table 5.—Number of sound onions harvested from soil, surface and seed treatments. Puyallup, Washington, 1956.[a]

Insecticide	Pounds per Acre	Total No. Sound Onions
Soil treatments		
Diazinon E.C.	10	304
Thimet E.C.	10	303
Thimet E.C.	5	292
Diazinon E.C.	5	283
Trithion W.P.	10	196
Trithion W.P.	5	191
Parathion E.C.	10	54
Sevin W.P.	5	19
Hercules AC-528 E.C.	5	13
Malathion E.C.	10	1
Seed treatments[b]		
Trithion W.P.	1 oz.	160
Diazinon W.P.	0 oz.	38
Parathion W.P.	1 oz.	34
Thimet-charcoal	1 oz.	31
Malathion W.P.	1 oz.	6
Two surface treatments		
Thimet E.C.	10	3
Parathion E.C.	10	2
Malathion E.C.	10	1
Diazinon E.C.	10	0
Trithion W.P.	10	0
One surface treatment		
Diazinon E.C.	10	2
Thimet E.C.	10	1
Malathion E.C.	10	1
Trithion W.P.	10	1
Parathion E.C.	10	1
Chlorthion E.C.	5	1
Guthion E.C.	5	1
Parathion E.C.	5	0
Shell O.S. 1836 E.C.	5	0
Malathion E.C.	5	0
Phosdrin E.C.	5	0
Methyl parathion E.C.	5	
Untreated		1
Untreated		0

[a] Onions planted July 10, 1956 and harvested September 10–14, 1956.
[b] Amounts given in ounces of actual ingredient per 1 lb. of seed.

Table 6.—Plant emergence and number of sound onions harvested from furrow treatments. Puyallup, Washington, 1957.[a]

Insecticide	Pounds per Acre	Plant Emergence: Total No.	Plant Emergence: Analysis	Sound Onions Harvested: Total No.	Sound Onions Harvested: Analysis
Untreated		606	gf	4	a
Untreated		626	hgf	13	ab
Dipterex granules	5.0	677	ihg	17	ab
Dipterex granules	2.5	734	i	22	ab
Endrin granules	2.5	650	hg	43	ab
Bayer 19639 granules	5.0	255	a	54	bc
Endrin granules	5.0	667	ihg	89	cd
Bayer 19639 granules	2.5	436	d	124	d
Thimet granules	5.0	309	ab	219	e
Thimet granules	2.5	353	bc	223	e
Thimet charcoal	5.0	323	b	242	e
Thimet charcoal	2.5	421	cd	263	e
Diazinon dust	5.0	528	e	416	f
Diazinon granules	5.0	527	e	418	f
Diazinon dust	2.5	565	fe	445	gf
Trithion W.P.	2.5	678	ihg	489	hg
Trithion W.P.	5.0	641	hg	516	h
Ethion dust	5.0	655	hg	531	ih
Diazinon granules	2.5	644	hg	565	ji
Ethion dust	2.5	698	ih	592	j

[a] Onions planted and tested July 16, 1957; harvested October 28, 1957.

Table 7.—Plant emergence and number of sound onions harvested from furrow treatments. Puyallup, Washington, 1957.[a]

Insecticide	Pounds per Acre	Plant Emergence		Sound Onions Harvested	
		Total No.	Analysis	Total No.	Analysis
Untreated		726	fe	3	a
Dipterex granules	2.5	708	fed	16	a
Endrin granules	1.25	690	ed	17	a
Untreated		689	ed	33	ab
Dipterex granules	1.25	738	f	39	ab
Endrin granules	2.5	786	g	65	b
Bayer 19639	2.5	531	b	115	c
Bayer 19639	1.25	632	c	125	c
Thimet charcoal	1.25	537	b	283	d
Thimet granules	2.5	424	a	300	de
Thimet granules	1.25	549	b	329	ef
Thimet charcoal	2.5	556	b	351	f
Trithion W.P.	2.5	683	d	420	g
Trithion W.P.	1.25	727	fe	421	g
Diazinon dust	2.5	559	b	472	h
Diazinon granules	2.5	570	b	485	h
Ethion dust	1.25	746	gf	518	ih
Diazinon granules	12.5	641	c	537	i
Ethion dust	2.5	685	ed	537	i
Diazinon dust	12.5	693	ed	549	i

[a] Onions planted June 28, 1957; harvested October 31, 1957.

surface, or furrow treatment. Isodrin was the most effective of the chlorinated hydrocarbons, particularly when used in the furrow. When the production of isodrin was discontinued, endrin was evaluated. It was hoped that endrin, a stereoisomer of dieldrin, would give results comparable to isodrin, the stereoisomer of aldrin.

Finlayson *et al.* (1958) reported that endrin employed as a furrow treatment at 2 pounds actual per acre gave approximately 25% control in the Puyallup area. They reported 100% damaged onions with similar treatments of heptachlor and dieldrin.

Endrin used as a seed treatment at 3, 4.5, or 6 ounces of actual material per pound of seed gave significantly better results than endrin used as a soil treatment at 5 pounds actual per acre. Endrin as a seed treatment at the 6-ounce rate gave significantly better results than at 3 or 4.5 ounces. There was no significant difference between the 3- and 4.5 ounce rates. Endrin seed treatments gave good control for several weeks, but rapidly became ineffective as the season advanced.

None of the organic phosphates tested gave economic control as seed, surface, or total soil treatments. However, the soil treatments proved to be an effective way of screening the organic phosphates for onion maggot control under field conditions. Thimet, ethion, Diazinon and Trithion were promising when tested in this manner. In later experiments these same toxicants were the most effective and gave excellent control when applied as furrow treatments.

Because the testing of organic phosphates as total soil treatments in 1956 failed to produce effective control, the most promising of these compounds were tested as furrow treatments in 1957 and 1958. In addition, endrin and several organic phosphates not previously evaluated were included. Bayer 21/199, ethion, Guthion, Trithion, and Diazinon gave economic control at 1 pound per acre. However, with the exception of Diazinon, significant increases in yield were obtained at the 2-, 3-, and 5-pound dosages. Diazinon at higher rates was phytotoxic so that increased dosages reduced the yield.

In a single experiment V-C 13[3] at 2 and 4 pounds gave outstanding control so that further work with this compound would be justified.

Malathion, Dipterex, Perthane, Bayer 19639, Dow

[3] *O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate.

Table 8.—Plant emergence yield in pounds, and number of sound onions harvested from furrow treatment with granulated insecticides. Puyallup, Washington, 1957.[a]

Insecticide and Per Cent Concentration	Pounds per Acre	Sound Onions at 30 Days		Weight of Onions		Sound Onions Harvested	
		Total No.	Analysis[a]	No. of Pounds	Analysis	Total No.	Analysis
Untreated		78	a	0.3	a	3	a
Malathion 10	2	512	e	0.5	a	6	a
Dipterex 2.5	2	562	ef	0.4	a	10	a
Thimet 16	4	156	a	19.5	b	96	b
Endrin 5	2	290	d	15.5	e	178	bc
Endrin 5	6	241	bc	20.3	de	163	bcd
Endrin 5	4	219	b	14.8	bc	119	bcd
Thimet 16	2	285	cd	24.7	cd	141	cde
Endrin 25	4	588	ef	29.8	f	291	de
Endrin 25	2	540	ef	29.7	f	268	e
Parathion 5	2	574	fg	20.7	bc	122	f
Parathion 5	4	618	g	20.7	b	100	f
Diazinon 8	2	528	ef	88.7	g	538	g
Guthion 2.5	2	722	h	73.0	g	535	g

[a] Onions planted and treated May 20, 1957; harvested October 30, 1957.

Table 9.—The number of sound onions harvested from furrow treatments with granulated insecticides. Puyallup, Washington, 1957.[a]

Insecticide and Per Cent Concentration	Pounds per Acre	Non-Infested Onions	
		Total Number	Analysis
Untreated		29	a
Malathion 10	2	99	a
Dipterex 2.5	2	176	a
Thimet 16	4	223	a
Endrin 5	2	299	b
Endrin 5	6	302	b
Endrin 5	4	317	b
Thimet 16	2	460	c
Endrin 25	4	664	c
Endrin 25	2	772	d
Parathion 5	2	797	d
Parathion 5	4	1019	e
Diazinon 8	2	1037	e
Guthion 2.5	2	1447	f

[a] Onions planted and treated May 20, 1957; harvested August 12, 1957.

Table 10.—Number of sound onions harvested from furrow treatments. Puyallup, Washington, 1957.[a]

Insecticide	Pounds per Acre	Sound Onions Harvested	
		Total No.	Analysis
Untreated		14	a
Parathion granules	2	123	a
Parathion granules	4	188	a
Perthane dust	2	545	b
Bayer 19639 granules	2	580	b
Perthane dust	4	712	b
Guthion granules	2	1973	c
V-C 13 granules	4	2158	cd
Diazinon granules	2	2295	de
Bayer 21/199	2	2304	de
V-C 13 W.P.	2	2384	de
Bayer 21/199	4	2506	e

[a] Onions planted and treated July 25, 1957; harvested October 31, 1957.

Table 11.—Effect of insecticide treatments on plant emergence and number of sound onions harvested from furrow treatments. Puyallup, Washington, 1958.[a]

Insecticide	Active Ingredient (Lb. per Acre)	Plant Emergence		Sound Onions Harvested	
		Total No.	Analysis	Total No.	Analysis
Untreated		829	defghijklm	0	a
Dow ET-57 granules	3	9	a	1	a
Dow ET-57 granules	2	31	a	1	a
Untreated		651	cdef	5	a
Untreated		696	cdefgh	5	a
Dow ET-57	1	138	a	7	a
Kepone W.P.	1	1110	op	34	abc
Thiodan granules	1	874	efghijklmn	51	abcd
Thiodan granules	2	734	cdefghi	59	abcd
DDT granules	1	890	ghijklmno	62	abcde
Kepone W.P.	2	1050	mnop	82	abcde
Kepone W.P.	3	1029	klmnop	107	abcde
DDT granules	2	867	efghijklmn	124	abcde
Thiodan granules	3	748	defghi	127	abcdef
DDT granules	3	882	fghijklmno	133	abcdef
Sevin granules	1	810	defghijkl	162	cdef
Sevin granules	3	642	cde	181	def
Sevin granules	2	748	defghi	257	fg
Diazinon granules	3	514	bc	448	h
Trithion granules	1	797	bc	486	hi
Ethion granules	1	873	efghijklmn	509	hij
DDT-Ethion granules	1	775	defghij	517	hij
DDT-Ethion granules	2	807	defghijkl	539	hijk
Guthion granules	1	834	defghijklm	568	hijkl
Trithion granules	2	827	defghijklm	575	hijkl
Bayer 21/199 W.P.	1	1078	nop	577	hijkl
Diazinon granules	1	672	cdefg	611	ijklm
Trithion granules	3	826	defghijklm	621	jklmn
Diazinon granules	2	689	cdefgh	637	jklmn
Guthion granules	3	918	hijklmno	670	klmno
DDT-Ethion granules	3	922	hijklmno	680	lmno
Ethion granules	2	961	ijklmno	693	lmno
Guthion granules	2	860	efghijklmn	717	mnop
Ethion granules	3	891	ghijklmno	726	mnop
Bayer 21/199 W.P.	2	1213	p	836	p
Bayer 21/199 W.P.	3	1203	p	836	p

[a] Plots planted and treated May 6, harvested July 23, 1958.

ET-57, Thiodan DDT, Sevin,[4] Kepone,[5] and endrin were ineffective at all dosages used. Thimet gave good control but was extremely phytotoxic at all rates resulting in poor yields. Parathion at 2 and 4 pounds protected the onions for several weeks, then declined rapidly.

Since it appears that the success of organic phosphates in controlling the onion maggot depends on their performance as furrow treatments, the matter of phytotoxicity is important. Granulated Dow ET-57 was severely phytotoxic at all rates so that only a few seeds germinated. Highly significant reductions in germination occurred when wettable powder formulations of Bayer 19639 and wettable powder and granules of Thimet were used at the 1.25, 2.5, and 5 pound rates. Diazinon dust and granules at 1.25, 2.5, and 5 pounds significantly decreased germination. Germination in the Bayer 21/199 and Kepone treatments was significantly better than in the untreated plots. Wettable powder of 25% Trithion, 5% ethion dust, granulated 25% endrin, and granulated Dipterex 2.5% did not reduce germination at the 1.25-, 2.5-, or 5-pound dosages.

Reference Cited

Finlayson, D. G., H. H. Crowell, A. J. Howitt, D. R. Scott, and A. J. Walz. 1958. Experiments on chemical control in onions grown from seed in British Columbia, Idaho, Oregon, and Washington in 1955 and 1956 (Unpublished data).

[4] 1-naphthyl N-methyl carbamate.

[5] 1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one.

Dieldrin as a Permanent Mothproofing Agent[1]

R. J. NORTON,[2] A. CAPELLE,[3] and S. D. EDMOND[3,4]

Abstract

Samples of Botany flannel standard test fabric and standard blanket fabric, dieldrin-treated and untreated, dyed and undyed, manipulated far in excess of pertaining American Society for Testing Materials (ASTM) requirements and unmanipulated, were subjected to 28 days of feeding by black carpet beetle larvae (*Attagenus piceus* (Oliv.)) in accordance with ASTM Designation: D 582-54 to determine the efficacy and durability of dieldrin as a permanent mothproofing agent for woolens. Five replicate test strips were used for each test increment, with 10 replicate test strips being used for each untreated manipulated and untreated unmanipulated increment to establish base-line feeding potentials.

Dieldrin-treated fabrics, dyed and undyed, manipulated far in excess of pertaining ASTM requirements and unmanipulated, were protected against black carpet beetle larval feeding throughout the duration of the test period. Dieldrin-treated fabrics which, prior to feeding exposure, had been subjected to 20 washings, 25 drycleanings with Stoddard solvent, 25 Hoffman pressings, 5 Stoddard drycleanings plus wet cleaning, 5 sea water saturations, 5 acid or 5 alkaline perspiration saturations, 100 hours in a Fadeometer, or in excess of the ASTM standard abrasion test, were protected against black carpet beetle larval feeding. Such manipulations did not appear to affect the protectant efficacy or durability of the dieldrin treatment. All untreated manipulated and untreated unmanipulated fabrics were destroyed by severe warp and severe nap feeding.

The Diels-Alder reaction, essentially a reaction between a conjugated diene and an olefinic carbonyl compound, has given rise to many widely used chlorinated hydrocarbon insecticides. Among these, dieldrin is widely used (McCauley & Sun 1954) in diverse fields of foreign and domestic markets. As early as 1949 Kearns *et al.* reported, among other data, that dieldrin was significantly effective against larvae of both black carpet beetle (*Attagenus piceus* (Oliv.)) and webbing clothes moth (*Tineola bisselliella* (Hum.)) in protection of wool samples. In 1955 Lipson and Hope reported that application of 0.05% dieldrin based on the weight of the fabric gave good protection of wool against feeding damage by larvae

[1] Investigations conducted under a research grant sponsored by Shell Chemical Corporation, New York, N. Y. and in cooperation with the New Hampshire Agricultural Experiment Station, Durham, N. H. Partial cost of publication of this paper was met by the Crop Protection Institute, Inc., Durham, N. H. Accepted for publication August 25, 1958.

[2] Director, Crop Protection Institute, Inc., Durham, N. H.

[3] Research Associate, Crop Protection Institute, Inc., Durham, N. H.

[4] The authors gratefully acknowledge the cooperation and assistance of personnel of Shell Chemical Corporation in the pursuit of these studies

of both clothes moth and carpet beetle and that the effectiveness of the dieldrin was fast to solvent extraction and washing. In 1956 the Wool Industries Research Association reported that dieldrin applied to wool from aqueous dispersions in dyeing or wet finishing was resistant to washing or drycleaning. Dieldrin subsequently became widely used in Great Britain and Australia for commercial protection of woolen fabrics. Data accumulated during the interim indicated that when properly applied dieldrin gave promise of being a permanent protectant with reasonable assurance that the duration of protection might be equivalent to the normal life of the woolen product.

These investigations were undertaken to determine the efficacy of dieldrin as a mothproofing agent and to ascertain to what extent the protection imparted by dieldrin would resist removal procedures of laundering, solvent extraction and other service tests of the current American Society for Testing Materials (ASTM) specifications for permanent mothproofing.

MATERIALS.—Botany flannel, specified by ASTM Designation: D 627–54 as official test fabric, and standard blanket fabric supplied by Faribault Woolen Mills, Faribault, Minn., were treated by Reider Associates, Reading, Pa., and Faribault Woolen Mills, respectively, with a dieldrin emulsion made from a xylene-base containing 1.5 pounds of dieldrin per gallon, added during the dye bath process. Both dyed and undyed samples were treated with dieldrin. All treated samples, together with comparable untreated samples, including both dyed and undyed fabrics, were then submitted to the National Institute of Drycleaning, Inc., Silver Spring, Md., for manipulation in accordance with specifications of ASTM Designation: D 627–54 and in accordance with extended additional testing proposed by the United States Department of Agriculture and Shell Chemical Corporation. Representative samples of each fabric, including dyed and undyed, treated and untreated, and manipulated and unmanipulated were then submitted directly to the Crop Protection Institute, Durham, N. H., for determination of the degree of protection achieved against feeding by black carpet beetle larvae in accordance with ASTM Designation: D 582–54. Comparable samples were submitted to the Shell Development Company for quantitative analysis to determine the dieldrin content.

TEST FABRICS.—ASTM Designation: D 627-54 specifies service test methods by which the permanency of protection by a given treatment may be determined. Modifications of this Designation used in these investigations (Mitchell & Williams 1958) in comparison with specifications of the ASTM Designation were as shown above.

Manipulation	*ASTM Designation: D627-54*	*Modifications Used for Dieldrin-treated Wool Tests*
Washing	5 times	20 times
Dry cleaning (Stoddard solvent)	5 times	25 times
Wet cleaning	5 times	5 times plus 5 dry cleanings
Hot pressing	5 times	25 times (Hoffman Press) plus 25 dry cleanings
Salt water	5 times	5 times
Perspiration—acid	5 times	5 times
Perspiration—alkaline	5 times	5 times
Light (Fadeometer)	40 hours	100 hours
Abrasion	2000 revolutions	Until nap removed

The above modifications represent the maximum number of respective service tests through which the several fabrics were manipulated. Although pertaining data are not herein reported, all fabrics were processed through manipulation increments from the ASTM minima through the modification maxima above indicated. Samples were removed and submitted for both feeding tests and chemical analyses after 5, 10, 15, and 20 washings; after 5, 10 15, 20, and 25 drycleanings; after 5, 10, 15, 20, and 25 Hoffman pressings; and after 20, 40, and 100 hours in the fadeometer.

The following Botany Flannel Official Test Fabrics (OTF) and Standard Blanket Fabrics (SBF), dieldrin treated and untreated, dyed and undyed, manipulated in excess of pertaining ASTM requirements and unmanipulated, are herein considered with tabular designation indicated:

Test Fabric	*Tabular Designation*
Official Test Fabric, dyed olive drab, dieldrin treated	OTF OD dieldrin
Official Test Fabric, dyed orange, dieldrin treated	OTF orange dieldrin
Official Test Fabric, undyed, dieldrin treated	OTF dieldrin
Standard Blanket Fabric, dyed blue, dieldrin treated	SBF blue dieldrin
Official Test Fabric, undyed, no dieldrin	OTF
Official Test Fabric, dyed olive drab, no dieldrin	OTF OD
Official Test Fabric, undyed, no dieldrin	OTF
Standard Blanket Fabric, dyed blue, no dieldrin	SBF blue
Standard Blanket Fabric, dyed blue, no dieldrin	SBF blue
Standard Blanket Fabric, undyed, no dieldrin	SBF

PROCEDURE.—All test fabrics were subjected to 28 days of feeding by black carpet beetle larvae, utilizing 10 larvae of 5.0 mg. average weight per individual feeding strip and five replicates for each test increment. Base-line feeding potentials were established by utilizing 10 replicated feeding tests of unmanipulated treated and untreated test increments. All feeding tests herein reported were conducted simultaneously under controlled conditions of $80° \pm 5°$ F. and $60 \pm 5\%$ relative humidity.

RESULTS.—Upon completion of 28 days of feeding all test units were examined for visual evidence of damage to the test fabric, milligrams of larval excrement per test strip, and number of living larvae. The results are presented in table 1. Visual damage to test fabrics was measured in terms of warp and nap feeding. Damage was recorded as warp feeding if either the woof or warp thread or both had been sheared by larval feeding. If a test strip showed two or more holes (warp damage), the damage was recorded as severe. Nap feeding was measured with a platinum loop calibrated to accurately measure an area of 0.50% of the test strip surface. Nap feeding was recorded as severe if the total damaged area exceeded 10% of the surface area of the test strip. Where nap feeding was of an

Table 1.—Feeding by black carpet beetle larvae on dieldrin-treated woolen fabrics.

Test Fabric	Manipulation	Visual Damage		Excrement (Mg.)	Living Larvae	Percentage Protection
		Warp	Nap			
OTF OD dieldrin	None	0	0	0.76	6.7	97.1
OTF orange dieldrin	None	0	0	0.34	3.5	98.7
OTF dieldrin	None	0	0	0.57	6.3	97.8
SBF blue dieldrin	None	0	0	0.59	7.1	97.7
OTF OD	None	Severe	Severe	22.39	10.0	15.1
SBF blue	None	Severe	Severe	23.53	10.0	15.1
SBF	None	Severe	Severe	24.56	9.9	6.9
OTF	None	Severe	Severe	26.38	9.9	0
OTF OD dieldrin	20 washings	0	< .14%	0.64	9.2	97.6
OTF orange dieldrin	20 washings	0	0	1.42	8.6	94.6
OTF dieldrin	20 washings	0	0.3%	0.70	10.0	97.3
SBF blue dieldrin	5 washings	0	0	1.02	6.8	96.2
OTF	20 washings	Severe	Severe	22.62	10.0	14.6
OTF OD	20 washings	Severe	Severe	23.06	9.6	12.6
OTF OD dieldrin	25 dry-cleanings with Stoddard solvent	0	0	0.70	8.2	97.3
OTF orange dieldrin	25 dry-cleanings with Stoddard solvent	0	0	0.70	7.0	97.3
OTF dieldrin	25 dry-cleanings with Stoddard solvent	0	0	0.50	5.0	98.1
SBF blue dieldrin	10 dry-cleanings with Stoddard solvent	0	0	1.00	8.2	96.2
OTF	25 dry-cleanings with Stoddard solvent	Severe	Severe	27.32	10.0	0
OTF OD	25 dry-cleanings with Stoddard solvent	Severe	Severe	20.90	10.0	20.8
OTF OD dieldrin	25 dry-cleanings with Stoddard solvent plus Hoffman pressing	0	0	1.08	10.0	95.9
OTF orange dieldrin	25 dry-cleanings with Stoddard solvent plus Hoffman pressing	0	0	0.88	9.8	96.7
OTF dieldrin	25 dry-cleanings with Stoddard solvent plus Hoffman pressing	0	1.9%	0.92	10.0	96.6
OTF	25 dry-cleanings with Stoddard solvent plus Hoffman pressing	Severe	Severe	13.94	6.6	52.8
OTF OD	25 dry-cleanings with Stoddard solvent plus Hoffman pressing	Severe	Severe	17.96	9.6	31.1
OTF OD dieldrin	5 Stoddard dry-cleanings plus wet cleaning	0	1.8%	0.92	10.0	96.5
OTF orange dieldrin	5 Stoddard dry-cleanings plus wet cleaning	0	0	0.54	6.4	97.9
OTF dieldrin	5 Stoddard dry-cleanings plus wet cleaning	0	0	0.50	9.2	98.1
OTF	5 Stoddard dry-cleanings plus wet cleaning	Severe	Severe	21.06	10.0	20.1
OTF OD	5 Stoddard dry-cleanings plus wet cleaning	Severe	Severe	21.50	10.0	18.5
OTF OD dieldrin	5 wet cleanings plus 5 dry-cleanings with Stoddard solvent plus Hoffman pressing	0	0	1.00	7.8	96.2
OTF orange dieldrin	5 wet cleanings plus 5 dry-cleanings with Stoddard solvent plus Hoffman pressing	1 Hole	<0.28%	1.16	6.8	95.6
OTF dieldrin	5 wet cleanings plus 5 dry-cleanings with Stoddard solvent plus Hoffman pressing	0	<1.5%	0.76	8.8	97.1
OTF	5 wet cleanings plus 5 dry-cleanings with Stoddard solvent plus Hoffman pressing	Severe	Severe	19.10	9.4	27.6
OTF OD	5 wet cleanings plus 5 dry-cleanings with Stoddard solvent plus Hoffman pressing	Severe	Severe	20.28	8.6	23.2
OTF OD dieldrin	5 sea water saturations	0	0	1.42	2.0	94.6
OTF orange dieldrin	5 sea water saturations	0	0	0.90	1.8	96.6
OTF dieldrin	5 sea water saturations	0	0	0.74	0.0	97.1
OTF	5 sea water saturations	Severe	Severe	24.18	10.0	8.3
OTF OD	5 sea water saturations	Severe	Severe	16.46	10.0	37.7
OTF OD dieldrin	5 acid perspiration saturations	0	0	0.86	4.4	96.7
OTF orange dieldrin	5 acid perspiration saturations	0	0	0.98	0.2	96.3
OTF dieldrin	5 acid perspiration saturations	0	0	0.94	4.4	96.4
OTF	5 acid perspiration saturations	Severe	Severe	28.26	10.0	0
OTF OD	5 acid perspiration saturations	Severe	Severe	30.78	10.0	0
OTF OD dieldrin	5 alkaline perspiration saturations	0	0	1.10	4.4	95.8
OTF orange dieldrin	5 alkaline perspiration saturations	0	0	0.88	0.4	96.7
OTF dieldrin	5 alkaline perspiration saturations	0	0	0.68	0.6	97.4
OTF	5 alkaline perspiration saturations	Severe	Severe	41.80	10.0	0
OTF OD	5 alkaline perspiration saturations	Severe	Severe	25.64	10.0	2.8
OTF OD dieldrin	100 hours in fadeometer	0	0	1.10	8.6	95.8
OTF orange dieldrin	100 hours in fadeometer	0	0	0.88	1.2	96.7
OTF dieldrin	100 hours in fadeometer	0	0	1.16	7.6	95.6
OTF	100 hours in fadeometer	Severe	Severe	24.80	8.8	6.0
OTF OD	100 hours in fadeometer	Severe	Severe	17.54	9.6	33.5
OTF OD dieldrin	Abrasion until nap removed	0	0	0.86	5.8	96.7
OTF orange dieldrin	Abrasion until nap removed	0	0	0.82	3.4	96.9
OTF dieldrin	Abrasion until nap removed	0	<0.14%	0.96	6.2	96.4
OTF	Abrasion until nap removed	Severe	Severe	28.64	9.0	0
OTF OD	Abrasion until nap removed	Severe	Severe	21.92	9.6	16.9

extremely minor degree, the amount of damage inflicted was numerically recorded as a percentage of the total surface area of the test strip.

Percentage protection of the respective fabrics was computed on the basis of the excrement weight method (ASTM Designation: D 582-54) utilizing average milligrams of larval excrement on test strips of untreated, undyed, and unmanipulated official test fabric as a base of reference. Prior data (unpublished) indicate that texture and dyes may influence the degree of carpet beetle larval feeding, thus ruling out the use of fabrics for control reference which, though not treated with a carpet beetle deterrent composition, have, nevertheless, been subjected to various manipulations and/or contain various dye compositions.

DISCUSSION.—Warp and nap damage inflicted on the untreated unmanipulated and untreated manipulated controls was severe and within the normal range of feeding for such investigations conducted at these laboratories. All dieldrin-treated fabrics, manipulated far in excess of pertaining ASTM requirements and unmanipulated, exhibited protection against black carpet beetle larval feeding. In some instances nap feeding was detected on treated fabrics. The extent of such feeding, however, was of an extremely minor degree and not considered significant.

Extreme manipulation of dieldrin-treated fabrics did not appear to negate the deterrent efficacy of the dieldrin. Samples of dieldrin-treated standard test fabric, dyed and undyed, which had been subjected to 20 washings, 25 drycleanings with Stoddard solvent, or 25 Hoffman pressings were not damaged by larval feeding. Samples subjected to 100 hours in the fadeometer were free of larval feeding. Blanketing subjected to 10 drycleanings with Stoddard solvent was not damaged by larval feeding.

ASTM Designation: D 582-54 states (par. 6(b) Note 2): "In the absence of visual damage, the specimen shall be considered satisfactorily resistant to carpet beetles if a quantity of excrement not over 6 mg. is deposited by the larvae, provided that under the same conditions a quantity of more than 25 mg. of excrement is deposited on the control specimens, and where the sample shows at least 75% protection, based on the excrement deposited on the untreated fabric or the standard test fabric as against the excrement on the treated fabric." By such calculation average protection afforded to all dieldrin-treated fabrics is 96.7%. This level of protection is 21.7% greater than required by the foregoing ASTM Designation. Of all dieldrin-treated fabrics considered, two only, orange dyed dieldrin-treated official test fabric with 20 washings and olive drab dieldrin-treated official test fabric with five sea water saturations, afforded protection less than 95% with actual readings of 94.6% each.

CONCLUSIONS.—On the basis of data reported herein dieldrin-treated fabrics were protected against 28 days of feeding by black carpet beetle larvae.

Dieldrin-treated fabrics which, prior to feeding exposure, had been manipulated through 20 washings, 25 drycleanings with Stoddard Solvent, 25 Hoffman pressings, 5 Stoddard drycleanings plus wet cleaning, five sea water saturations, five acid or five alkaline perspiration saturations, 100 hours in a fadeometer, or in excess of the ASTM standard abrasion test, were protected against black carpet beetle larval feeding.

Such manipulations do not appear to affect the protectant efficacy or durability of the dieldrin treatment.

REFERENCES CITED

American Society For Testing Materials. 1956. Tentative method of test for resistance of textile fabrics and yarns to insect pests. ASTM Standards on Textile Materials. Designation: D 582-54: 119–24.

American Society for Testing Materials. 1956. Evaluating compounds designed to increase resistance of fabrics and yarns to insect pests. ASTM Standards on Textile Materials. Designation: D 627-54: 125–28.

Kearns, C. W., C. J. Weinman, and G. C. Decker. 1949. Insecticidal properties of some new chlorinated organic compounds. Jour. Econ. Ent. **42:** 127–34.

Lipson, M., and R. J. Hope. 1955. Mothproofing of wool; II. Dieldrin and related compounds. Nature. **171:** 599.

McCauley, W. E., and Y. P. Sun. 1954. Dieldrin. Soap and Chemical Specialties. **30:** 171.

Mitchell, L. E., and A. Williams. 1958. Permanent mothproofing of wool with dieldrin. Paper presented at 133rd meeting of American Chemical Society, San Francisco, California.

Wool Industry Research Association. 1956. The mothproofing properties of dieldrin. W. I. R. A. Bull. **18**(4): 136–42.

SUPPORT AA OF YOUR SOCIETY'S PUBLICATIONS:

It will strengthen *your* Society's position in representing *your* profession.

Factors Affecting the Fumigation of Food Commodities for Insect Control[1]

WALTON B. SINCLAIR and DAVID L. LINDGREN, *University of California Citrus Experiment Station, Riverside*

ABSTRACT

The experimental data presented in this report indicate that the problem in commercial fumigation is to treat the food commodities with a fumigant at the lowest concentration that will sterilize them against insect infestations and at the same time expose the commodity to a concentration sufficiently low to prevent physical injury so that its commercial value will not be decreased. A fumigant that is adequate for sterilizing food commodities against insect infestations should have the following additional qualities: (1) The fumigant must be a gaseous substance or a liquid compound that is easily converted to the gaseous state and maintained in that condition during the exposure period. (2) A liquid fumigant must have a sufficiently low boiling point so that it can be easily converted and maintained in the gaseous state at room temperature. (3) It is usually advantageous for a fumigant to have a low molecular weight, for the lower the molecular weight of a fumigant the greater is the diffusion rate. (4) In general it is preferable that a fumigant be insoluble in water, since those that are highly soluble in water are usually highly sorbed by the treated commodities, and the highly sorptive rates of a commodity for a fumigant are positively correlated with an excessive amount of residue. (5) Fumigants must not leave within the commodity an excess amount of toxic residue. (6) If fresh fruits are the commodities that are being treated the fumigant must not cause external injury such as pitting and blackening of the peel. (7) Fumigants must not cause internal injury such as discoloration and darkening of the edible portion of the fruit. (8) They must not prevent uniform maturation and softening of the fruit during storage. (9) The treatment must not produce or cause a development of off-flavor. (10) The treatment must not shorten the storage or shelf-life of the commodity.

The fumigation process is a method that has proved practical and efficient in sterilizing food commodities against insect infestations. To use this process efficiently, it is important to determine the physical and chemical properties and the reactions of both the fumigant and the material being fumigated in order to determine the most desirable fumigant for commodity treatment of a food material. For more than a decade special interest has been paid to the screening and studying of many fumigants for the specific purpose of determining the concentration time-exposure curves of those fumigants which citrus fruit, avocados, and other subtropical fruits can tolerate without injury, and at the same time applying these relationships to those compounds which have already been proved to be toxic to eggs and larvae of the various fruit flies.

During this extended period of research much experimental data has accumulated on the numerous factors which affect the efficient and practical treatment of a food commodity by a given fumigant. The choice of a gas for the fumigation of food commodities and biological materials depends upon (1) the physical and chemical properties of the gas, (2) the toxicity of the gas to the infesting insect, and (3) the physiological and chemical response of the material being fumigated. A thorough knowledge of these factors is very important for successful fumigation procedure with a given gas. In fumigating living tissue against infestation, the margin of safety is usually small between the concentration of the fumigant required to kill the insect and that sufficiently low to avoid injury to the tissues and toxic residues in the tissues in such concentrations as to be harmful when the material is consumed as food.

The main objective of this paper, therefore, is to present a report on the effect of physical and chemical properties of the gases on fumigation results, and, secondly, on the physical and chemical response of the fumigated material to the fumigation treatment. The factors affecting the toxicity of various fumigants to insect infestations have been discussed at length by Sun (1947) and will not be considered in this report.

BOILING POINT OF FUMIGANTS IN RELATION TO MOLECULAR WEIGHT.—The data in figure 1 show the relation of the boiling points of different fumigants to their molecular weights. The boiling points of these compounds increase with increasing molecular weights. All four of these compounds are liquid at room temperatures, but the compounds with the lower molecular weights have very high vapor pressures at ordinary temperatures and thus go very readily from the liquid to the gaseous state when placed inside a fumatorium. The higher the molecular weight of the fumigant, the lower the vapor pressure at room temperature, and, consequently, the longer the time required for a measured quantity to go from the liquid to the gaseous phase. Although methyl bromide is an excellent commercial fumigant, it does not fit into this relationship as it has a relatively high molecular weight (95) and a high vapor pressure associated with a low boiling point of 38° F. In practical experiments the fumigants with relatively high molecular weights, such as ethylene dibromide, are measured into a glass receptacle and placed on a hot plate inside the fumatorium to accelerate the evaporation of the liquid ethylene dibromide.

SPECIFIC GRAVITY OF FUMIGANT IN RELATION TO MOLECULAR WEIGHT.—The specific gravity of a fumigant is important because it shows the relative heaviness of the material in comparison with some other material taken as a standard, usually water. A specific gravity of 2.5, for example, means that a given material weighs 2.5 times as much as the same volume of water. The relationship of the specific gravities of certain fumigants to their

[1] Paper No. 1056, University of California Citrus Experiment Station, Riverside, California. Accepted for publication September 12, 1958. Partial cost of the publication of this paper was met by the University of California Citrus Experiment Station, Riverside.

The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

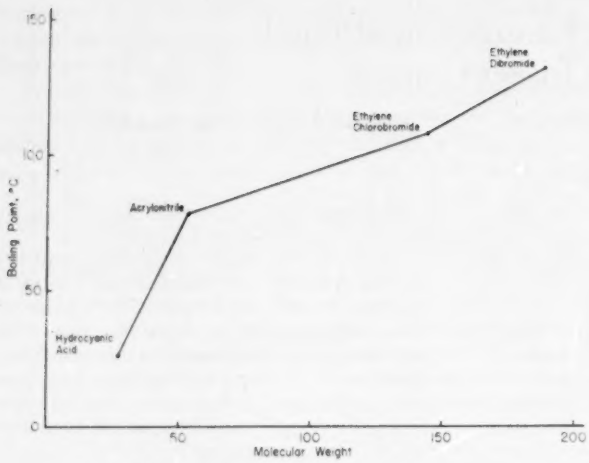


FIG. 1.—Relation of boiling points of various fumigants to their molecular weights.

molecular weights shows a significant positive correlation (fig. 2). All gases are miscible in all proportions and they mix spontaneously and rapidly, but not all gases diffuse at the same rate. When these fumigants are in the gaseous state their rates of diffusion are inversely proportional to the square roots of their densities. Since the densities of gases are proportional to their molecular weights, their rates of diffusion are inversely proportional to the square roots of their molecular weights. Hence, those fumigants which diffuse most readily are those with the lowest molecular weights. Under identical conditions, hydrocyanic acid (HCN), which has a much lower molecular weight than ethylene dibromide, will diffuse more rapidly than the latter.

TEMPERATURE.—Temperature affects the physical and chemical properties of gases to the extent that it is important to carry out a process, such as fumigation, at a known or fixed temperature. The volatility of a fumigant increases with rising temperature; this means that the diffusion rate is accelerated by an increase in temperature. During the fumigation of citrus and avocado fruits against fruit fly infestations, the rate of metabolism and consequently the respiratory activity of both the fruit flies and the commodities are significantly influenced, up to a certain limit, by changes in temperature. It follows then that the temperature is highly important in determining the effectiveness of a fumigant, owing to the fact that the rate of respiration is highly correlated with the rate at which insects absorb a fumigant. As a generalization the toxicity of a fumigant increases with increase in temperature.

The temperature of the commodity being fumigated has a marked effect on the amount of fumigant sorbed during an exposure period. The relation of preconditioning temperature to the amounts of HCN recovered from green Valencia oranges is shown in figure 3. Although no moisture was visible, it is possible that some moisture condensed on the fruits that had been preconditioned at the lower temperatures (that is, at 43°, 50°, or 65° F.) during the 40-minute fumigation at 75° F. in the fumatorium. HCN is readily soluble in water; it would therefore seem logical to conclude that the presence of the condensed moisture on the cooler fruits at least partially explains why more HCN was recovered from them than from the warmer fruits. On the other hand, the presence of a film of moisture, though not continuous, would tend to impede the entrance of HCN into the fruits; the total amount absorbed by the film and the fruit might thus be reduced.

Perhaps it would be more logical to conclude that the cool fruits absorbed more HCN than the warm fruits because of the increased solubility of gaseous HCN at the lower temperatures. This explanation appears to agree very well with the gas law, which states that the lower the temperature, the greater the solubility of the gas. The greater viscosity of the water in or on the fruits at the lower temperatures would, of course, not interfere with the foregoing explanation. Even at the end of the 40-minute fumigation period, the fruits preconditioned at 43° F. were noticeably cooler than those preconditioned at 80°. The amount of HCN recovered from the fruits preconditioned at 43° was approximately twice that recovered from the fruits preconditioned at 80° F. The data in figure 3 indicate that large differences in temperature between that of the fruit and that which existed in the fumatorium should be avoided. Although the data reported here were determined with HCN as a fumigant, the same results were obtained with both methyl bromide and ethylene dibromide.

PENETRATION AND SORPTION OF FUMIGANTS.—According to Shepard *et al.* (1937), the efficiency of a fumigant depends, among other things, on the vapor pressure, or the tendency to vaporize, inherent in the fumigant. At 25° C. (77° F.) water and chloropicrin have about the same vapor pressure, whereas ethylene dichloride has a pressure 3.4 times as great, carbon tetrachloride 4.9 times, and carbon disulfide 15.2 times. Those fumigants which normally exist as gases at the temperature of fumigation are not limited in this way. Their introduction into a given space depends upon the equipment used.

Roark & Nelson (1929) give the concentrations of various fumigants which can exist in vapor form in a 1000-cubic-foot fumigation chamber. The concentration of a fumigant to give a complete kill of an insect population should be below the limits which can exist as a vapor in a given volume.

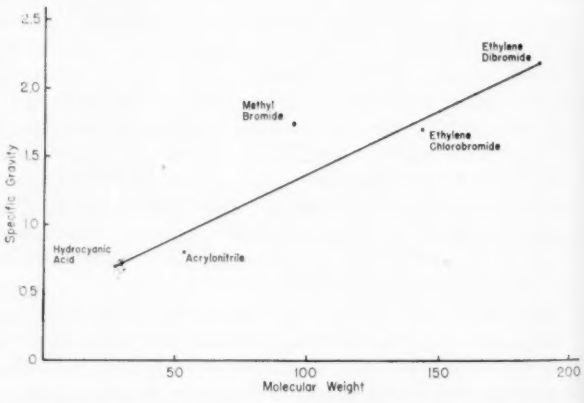


FIG. 2.—Relation of specific gravities of various fumigants to their molecular weights.

After the fumigant is vaporized, the problem is one of diffusion and mixing. Mass movement by air currents, as distinct from true molecular diffusion, is responsible to a large extent for equalizing the gas concentration in a chamber. To apply a liquid fumigant under pressure, or by injection as a hot gas, and to circulate the fumigant with a fan, aids in its uniform mixture with the air.

At the same time that the fumigant is mixed with the air by circulation, molecular diffusion takes place, although more slowly. At constant conditions of temperature and pressure, the rate of diffusion is inversely proportional to the square root of the density of the gas. Thus of two gases the lighter diffuses the more rapidly.

The extent to which molecular diffusion must be relied upon depends on the freedom with which mass movements of the atmosphere may take place. Diffusion is an important factor in the penetration of package goods by the gas, as well as in the loss of the fumigant through the walls of the chamber.

There are two factors which determine the penetration of a fumigant into the commodity being treated. The sorption of gases by solids is important in penetration processes. Although the rate of absorption is increased by rise of temperature, the amount absorbed when equilibrium is reached is diminished. Heat dissociates a sorption compound. This fact is demonstrated in the case of charcoal, where the gas absorbed at a low temperature is given off again on heating. The effect of temperature on the rate of sorption is found to be of the same order as that which it has on diffusion processes. Diffusion tends to carry the gas into the interstitial spaces, the rate depending upon the molecular weight of the fumigant, as just mentioned, and upon the size of the commodity being treated. The sorptive capacity of the product into which diffusion is in progress may affect the concentration of gas present. Not only are the spaces between the particles of the material being fumigated filled with a mixture of gas and air, but each particle attracts a layer of gas molecules to its surface. The sorptive capacity of a product must be satisfied before a constant concentration can be maintained in the surrounding atmosphere; therefore, it is important to know not only the relative toxicity of a fumigant and the rate of diffusion but also the sorptive capacity of the product that is being fumigated.

It has been determined experimentally that the size and composition of the load in the fumatorium has an effect on the concentration of the fumigant in the gas phase. A commodity that is being fumigated may sorb a fairly large amount of the gas, the degree of sorption depending on the character of the commodity and the quantity being fumigated, and on the fumigant used. The data in figure 4 show the influence of the load in the fumatorium on the decrease in concentration of the fumigant in the gas phase. Time-concentration curves are given for the four different fumigants.

The recovery of methyl bromide from an empty fumatorium and from fumatoria containing 9 and 19 boxes of fruit, respectively, is shown in figure 4A. The dosage in these experiments amounted to 2 pounds of methyl bro-

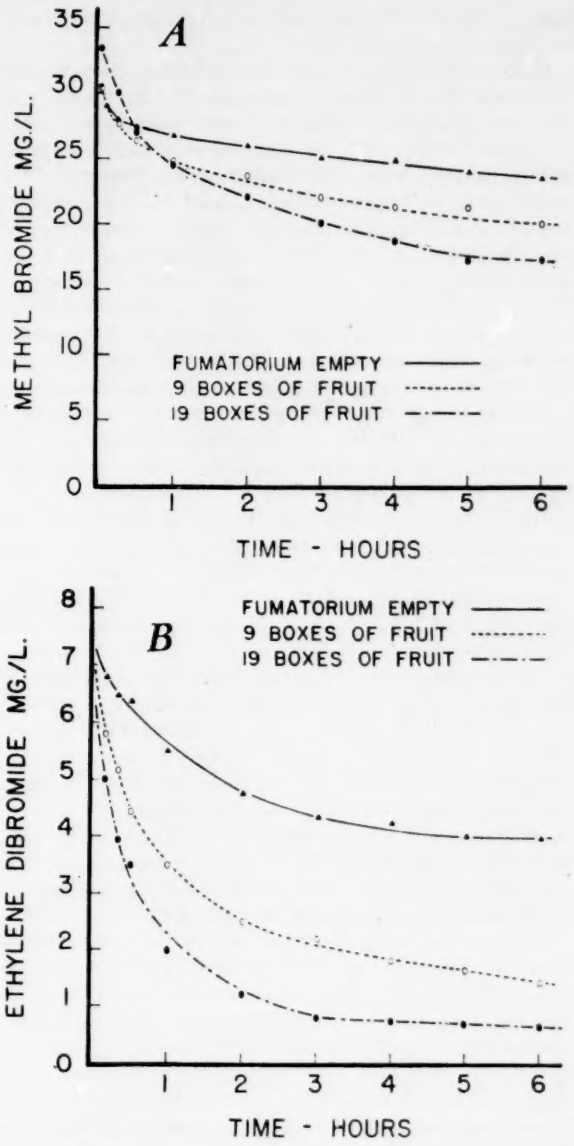


FIG. 4.—Relation between load (boxes of oranges) in a 100-cubic-foot fumatorium and concentration of fumigant in gas phase at various sampling intervals: (A) methyl bromide (dosage, 2 pounds per 1000 cubic feet; temperature, 70° F.); and (B) ethylene dibromide (dosage, 0.5 pound per 1000 cubic feet; temperature, 70° F.).

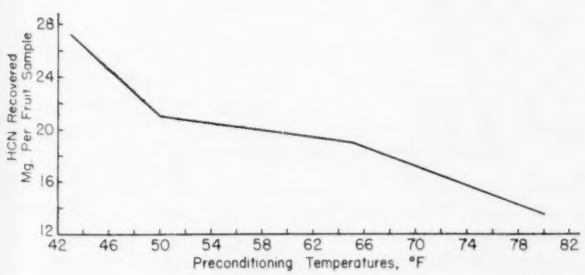


FIG. 3.—Amount of HCN recovered from samples of green Valencia orange fruits preconditioned overnight in the laboratory at 43°, 50°, 65° or 80° F. and fumigated in the laboratory fumatorium with 8 ml. HCN per 100 cu. ft.

mide per 1000 cubic feet, which is equivalent to 32 mg. per liter in the fumatorium.

In the empty fumatorium, the concentration of methyl bromide in the gas phase dropped from 32 mg. to 23.7 mg. per liter in the 6-hour exposure period, a decrease of 26%. In other words, 26% of the initial concentration of methyl bromide was sorbed by the exposed surface of the fumatorium in 6 hours. With a load of nine boxes of oranges in the fumatorium, the concentration of methyl bromide decreased from the initial amount to 20.2 mg. per liter during the 6-hour exposure period, a decrease of 37%. A similar curve for 19 boxes of oranges in the fumatorium showed that the concentration of methyl bromide in the gas phase decreased to 17.3 mg. per liter, a drop of 46% of the initial amount added to the fumatorium.

Similar curves for ethylene dibromide at a dosage of 0.5 pound per 1000 cubic feet are shown in figure 4B. This dosage, after correction for impurity in the fumigant, is equivalent to 7.68 mg. per liter in the fumatorium.

In the empty fumatorium, 48.5% of the initial concentration of ethylene dibromide was sorbed by the exposed surface in 6 hours. The curve for the empty fumatorium shows graphically that the concentration of ethylene dibromide fell from 7.68 to 3.96 mg. per liter during the time of exposure. With a load of nine boxes of oranges, the concentration of ethylene dibromide decreased approximately 82% during the 6-hour period, or to 1.38 mg. per liter. With 19 boxes of fruit, the amount of the fumigant decreased 92% in 6 hours. In other words, only 0.61 mg. per liter remained in the gas phase at the end of the fumigation period. Ethylene dibromide, at 0.5 pound per 1000 cubic feet, was therefore highly sorbed by the 9 and 19 boxes of oranges when it is considered that these two samples occupied only 18 and 38 cubic feet, respectively, of a total capacity of 100 cubic feet.

The curves for ethylene chlorobromide were drawn from data obtained at a dosage of 0.75 pound per 1000 cubic feet, which, after correction for impurity in the fumigant, is equivalent to 11.63 mg. per liter in the fumatorium (fig. 5A). The dosages used in these particular experiments are higher than those used for ethylene dibromide, but the slope and shape of the curves for these two closely related halogens are not significantly different. During the 6-hour period, the concentration in the empty fumatorium dropped from 11.63 to 4.88 mg. per liter, which is equivalent to a decrease of 58%. With loads of 9 and 19 boxes of oranges, the concentration in the chamber dropped to 2.22 and 1.10 mg. per liter, a decrease of approximately 81 and 91%, respectively. The difference between the amount sorbed by the empty fumatorium and that sorbed by the load gives the amount sorbed by the 9 and 19 boxes (23 and 32.5%, respectively).

The time-concentration curves for hydrogen cyanide are given in figure 5B. The dosage used in these experiments was 0.5 pound per 1000 cubic feet, which, after correction for the impurity in the fumigant, amounted to 7.38 mg. per liter.

That hydrogen cyanide is highly sorbed on surfaces is clearly demonstrated by noting the course of the curve for the empty fumatorium. The concentration in the gas phase decreased from 7.38 to 3.16 mg. per liter during the 6-hour period. In other words, 57% of the hydrogen cyanide was sorbed by the walls of the fumatorium. The high sorptive capacity of this fumigant is probably positively correlated with its solubility in water in all proportions. Moisture films on surfaces and biological materials generally increase the sorptive capacity of hydrogen cyanide from the gas phase during a fumigation. With loads of 9 and 19 boxes of fruit, the concentration fell to 1.03 and 0.70 mg. per liter, a decrease of 86 and 91% of the initial concentration, respectively. Although liquid hydrocyanic acid has a low boiling point (26° C.) and an exceedingly high vapor pressure at ordinary laboratory temperatures (654.4 mm. at 22° C.) the load of 19 boxes of oranges and

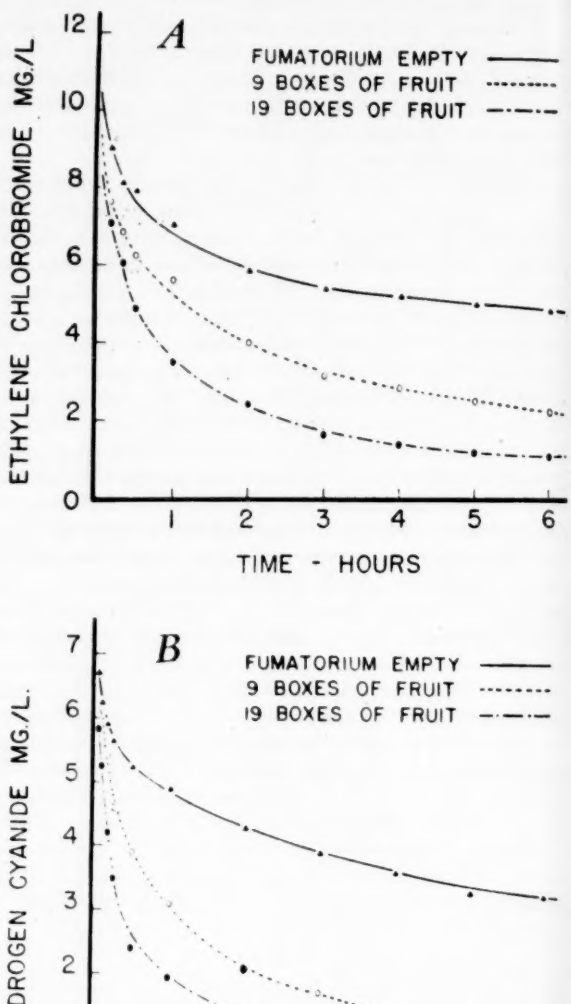


FIG. 5.—Relation between load (boxes of oranges) in 100-cubic-foot fumatorium and concentration of fumigant in the gas phase at various sampling intervals: (A) ethylene chlorobromide (dosage, 0.75 pound per 1000 cubic feet; temperature, 70° F.); and (B) hydrogen cyanide (dosage, 0.5 pound per 1000 cubic feet; temperature, 70° F.).

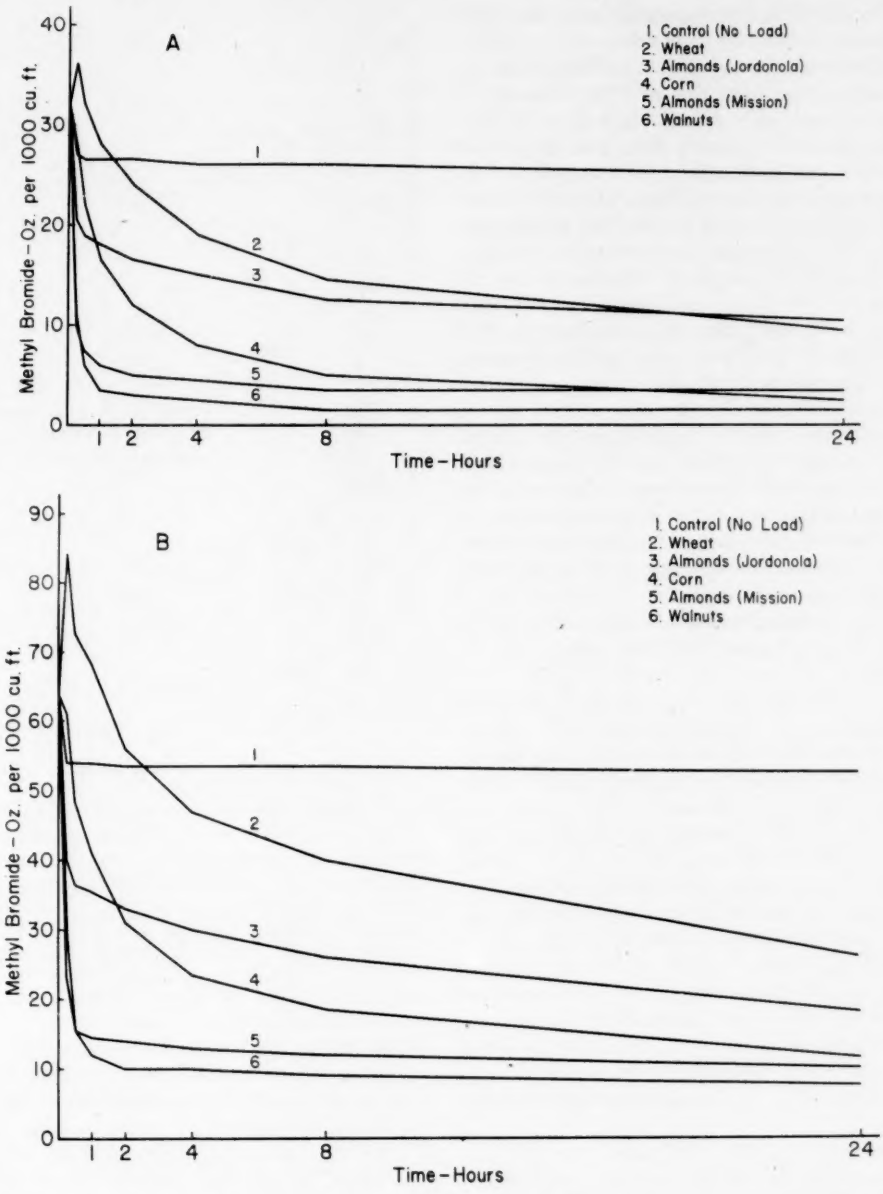


FIG. 6.—Effect of different food commodities on concentration of fumigant in the gas phase at various sampling intervals during a 24-hour fumigation period: (A) Dosage, 2 pounds methyl bromide per 1000 cu. ft.; (B) Dosage, 4 pounds methyl bromide per 1000 cu. ft.

the walls of the fumatorium sorbed nearly all the fumigant from the gas phase.

The rates of sorption of methyl bromide by various commodities at two different dosages and under well-defined conditions are strikingly shown in figures 6A and 6B. These fumigations were conducted in 53.3 liter recirculation-type gas-tight fumigation chambers. The commodities being fumigated occupied the same volume (about 90% of the fumigation chamber), but the weights varied as follows: wheat, 84.5 pounds; corn, 82.2 pounds; walnuts, 40.5 pounds; Mission almonds, 40 pounds; and Jordonola almonds, 36 pounds. The temperature at which the fumigations were conducted was 70° F., and the concentration of methyl bromide in the fumigation chamber was determined by the thermal conductivity method.

As each group of curves was determined under identical conditions, it is apparent that the decrease in concentration of the fumigant in the gas phase is due primarily to differences in the sorptive capacities of the various food commodities. As already noted, this difference is highly correlated with the physical and chemical characteristics and the composition of the treated commodity.

The most notable difference in these two sets of data (figs. 6A and 6B) is the relatively low sorptive capacity of wheat, in comparison with that of walnuts and Mission

variety almonds. These last two commodities sorbed a tremendous amount of the fumigant from the gas phase within the first hour of the fumigation period. Another interesting fact about the data is the striking difference in the initial rate of sorption of the fumigant by the two varieties of almonds: the Jordonola variety sorbed about half as much methyl bromide as the Mission variety.

From an inspection of the residue data from these fumigation experiments, it would appear that greater differences would occur in the total bromine sorbed between commodities and between levels of treatment than is manifested in table 1. It should be noted, however, that the samples were analyzed for bromine residues about 2 weeks after fumigation treatment. As methyl bromide has a relatively low boiling point and a high vapor pressure at room temperatures, the greater portion of the methyl bromide sorbed during the fumigation period would evaporate from the surface of the commodities during the 2-week interval between fumigation and analysis, thus leaving a bromine residue in the samples in the salt form. A noticeable difference in bromine residue between the 2- and 4-pound dosages occurred in only two samples, the corn and walnuts; the remaining three commodities, wheat, Mission almonds and Jordonola almonds, yielded about the same bromine residue at both dosages.

SORPTION OF VARIOUS FUMIGANTS BY EXCELSIOR PACKING MATERIAL.—We found early in our experiments on commodity treatments that the material in which a product was packed for shipment sorbed large quantities of the fumigant. Of the many kinds of packing materials available, excelsior in avocado shipping flats was chosen for these experiments. The sorption of the different fumigants by this material is shown in figure 7.

The concentration of methyl bromide used in this experiment (32 mg. per liter) was the same as that used in the fruit experiment. With a load of 50 flats of excelsior occupying 32 cubic feet in the fumatorium, the concentration of methyl bromide decreased from 32 to 17.1 mg. per liter, or approximately 46% (fig. 7A). This value includes the methyl bromide sorbed by both the exposed surface of the fumatorium (26%) and the excelsior packing material (20%).

Similar curves were constructed and reported for ethylene chlorobromide, ethylene dibromide, and hydrogen cyanide (fig. 7b). In comparing these curves, it should be noted that the concentration of hydrocyanic acid used in these experiments was different from that of

Table 1.—Bromine residue in various commodities after fumigation with methyl bromide.

Commodity	Per Cent Moisture	P.P.M. Bromine in Commodity After Fumigation with Methyl Bromide	
		2 Lb./1000 Cu. Ft.	4 Lb./1000 Cu. Ft.
Corn	12.6	7	26
Wheat	10.2	13	18
Almonds (Mission)	8.8	5	5
Almonds (Jordonola)	8.8	8	7
Walnuts	7.8	33	41

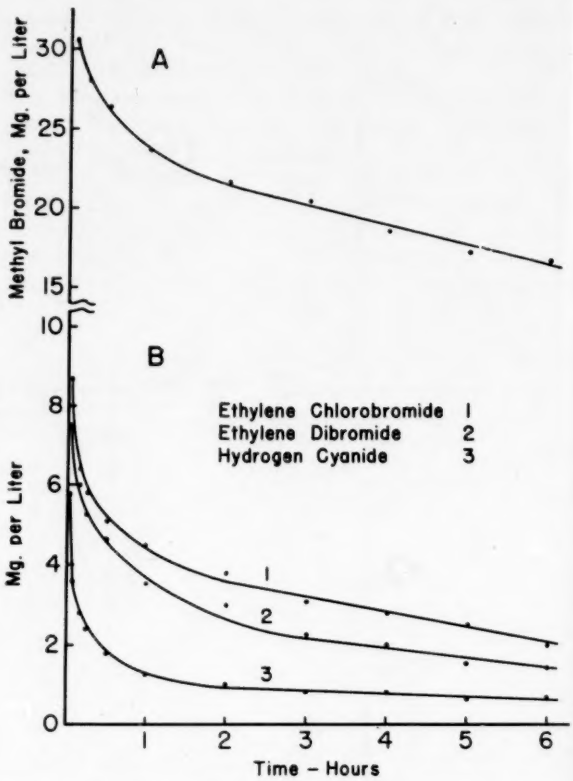


FIG. 7.—Relation between load (avocado shipping flats with excelsior) in a 100-cubic-foot fumatorium and concentration of fumigant in gas phase at various sampling intervals: (A) methyl bromide (dosage, 2 pounds per 1000 cubic feet); and (B) ethylene chlorobromide (dosage, 0.75 pound per 1000 cubic feet), ethylene dibromide (dosage, 0.75 pound per 1000 cubic feet), and hydrogen cyanide (dosage, 0.5 pound per 1000 cubic feet). All at a temperature of 70° F.

the other two fumigants. The data for the curves for both ethylene chlorobromide and ethylene dibromide were determined on the same concentration of fumigant. The excelsior material sorbed these two fumigants at about the same rate during the 6-hour exposure. During the fumigation period, the concentration of ethylene chlorobromide dropped from 11.63 to 2.02 mg. per liter, a decrease of approximately 83%; the concentration of ethylene dibromide fell from 11.52 to 1.49 mg. per liter, a decrease of 87%. The differences between these two series of values are not significant. With hydrocyanic acid, the curve descends very sharply, as shown by the fact that 81% of the initial concentration was sorbed by the excelsior material after 1 hour of exposure. During the total exposure period, the concentration fell from 7.38 to 0.73 mg. per liter, a sorption of approximately 90%.

EFFECT OF COMPOSITIONAL FACTORS AND DEGREE OF MATURITY ON THE SORPTION OF FUMIGANTS.—Considerable data have accumulated to show that the sorptive capacities of food commodities for a given fumigant are highly correlated with certain characteristics such as compositional and maturity factors of the treated commodities. In relation to maturity, Bartholomew *et al.* (1942) reported that green citrus fruits sorbed an average

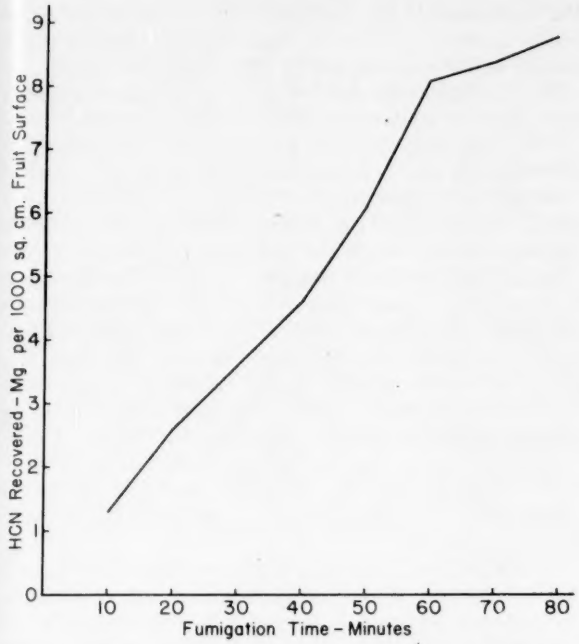


FIG. 8.—Relative amounts of HCN recovered from Valencia orange fruits fumigated at atmospheric pressure with 8 ml. of HCN for different lengths of time.

of 5.4 times as much HCN as mature fruits of equivalent surface area. This difference in the amount of HCN sorbed during a fumigation exposure is probably due to the differences in composition of the external surfaces of the peel of the green-immature and the yellow-mature Valencia oranges. As oranges change from a green to a completely yellow color during maturation, the external surface of the fruit also changes in chemical composition and morphological characteristics. By the time oranges have matured, the outer walls of the epidermal cells have become heavily cutinized, thus excessive transpiration is prevented, not only from these cells but also from the interior tissue. This heavily cutinized condition of the epidermal cell walls of the peel may well be the cause of the comparative lack of penetration and sorption of HCN by mature fruit.

EFFECT OF DIFFERENT EXPOSURE PERIODS ON THE SORPTION OF FUMIGANTS.—The longer the period of exposure to the fumigant, the greater the percentage mortality of the insect pest; but the effect of the fumigant on the tissues of the host must also be considered. The object of this series of experiments, therefore, was to determine the effect of the length of period of exposure on the fruits in the fumatorium, as indicated by the relative amounts of HCN recovered and the relative numbers of fruits injured. All fumigations in this series were made at atmospheric pressure. The amounts of sorbed HCN recovered from fumigated orange fruits, after different periods of exposure to a given concentration (8 ml.) of HCN, are shown in figure 8.

The data presented in figure 8 show that the increases in mean amounts of HCN sorbed (as indicated by the amounts recovered) were almost linear for each 10-minute period from 10 to 60 minutes; after that, the curve begins to flatten, and the amounts of HCN recovered were more variable. The decrease in recovery values which caused the curve to flatten may have occurred because the fruits had reached the peak of their rate of absorption by the end of the 60-minute period, or because the fruits, having become more heavily charged, had lost a larger percentage of the HCN before they could be put into the distillation flasks. The maximum capacity of the fruits for the absorption of HCN had not yet been reached, however, for although the curve begins to flatten after the 60-minute period, the trend is still upward for both the 70- and 80-minute exposure periods.

No fruits were injured at any of the exposure periods up to and including 40 minutes; but at the periods of 50, 60, 70, and 80 minutes, 3, 11, 14, and 15 fruits, respectively, were injured. Of these 43 fruits, 35 were slightly injured, 7 were moderately injured, and 1 (at the 80-minute exposure period) was badly injured.

The results of this series of experiments show, therefore, that the length of the period of exposure to the fumigant was an important factor in governing not only the amounts of HCN absorbed but also the extent of injury to the fruits.

SORPTION AT ATMOSPHERIC AND REDUCED PRESSURES WITH VARIOUS CONCENTRATIONS OF THE FUMIGANT.—Because vacuum fumigation, as well as fumigation at atmospheric pressure, is used for killing pests on living plant tissues, it seemed important to determine the relative effects of these two methods on the amounts of fumigant that Valencia oranges may sorb during a fumigation period, and on the extent of injury that may occur to the fumigated fruits. Hydrocyanic acid was the fumigant used in these experiments (fig. 9). The relative amounts of HCN absorbed were determined by measuring the amounts of HCN recovered from fruit samples fumigated at atmospheric pressure, and comparing these with the

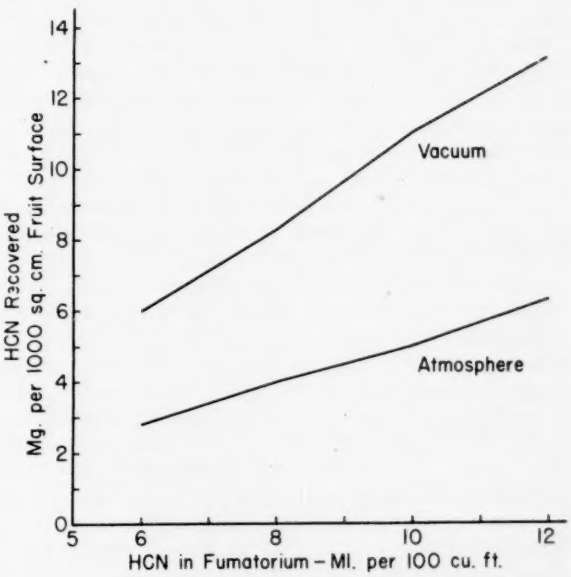


FIG. 9.—Relative amounts of HCN recovered from Valencia orange fruits fumigated at atmospheric pressure and at partial vacuum with various amounts of HCN.

amounts recovered from similar samples fumigated at a partial vacuum of approximately 26 inches. Four different concentrations of HCN (6, 8, 10, and 12 ml.) were used in the fumatorium in these experiments. The fumigation period was always 40 minutes.

The results presented in figure 9 show that as the concentrations of HCN in the fumatorium were increased, the amounts of HCN that could be recovered from the samples also increased. The plotted recovery values for fumigation under both atmospheric and reduced pressure conditions form almost straight lines, but values for atmospheric fumigation are a little less than half as great as those for partial vacuum fumigation, and the graph line for the latter is a little steeper than that for the former. Similar results have been obtained by Bhambhani & Blackith (1958) with certain insect species in which the capacity to sorb hydrocyanic acid increased as the total pressure was reduced.

The relative numbers of fruits injured by fumigation at atmospheric pressure and at partial vacuum, with the different dosages of HCN, were as follows:

	Number of Fruits Injured	
HCN Dosage (Ml.)	*At Atmospheric Pressure*	*At Partial Vacuum*
6	3	7
8	6	15
10	4	18
12	12	15

Each test included four samples of 10 fruits each. The data show that twice as many fruits were slightly injured at partial vacuum as at atmospheric pressure, and that five fruits were moderately or badly injured at partial vacuum and none at atmospheric pressure.

WATER-SOLUBLE FUMIGANTS.—Commercial fumigants vary in their degree of solubility in water. It is well known that the total solubility of a nonpolar gas (such as N_2, H_2, and CH_4) in a liquid is very small at room temperature. According to Henry's Law, the weight of a slightly soluble gas dissolved in a given quantity of solvent is nearly directly proportional to the partial pressure of that gas. A gas which is distinctly polar and which is in contact with a liquid, exhibits strong attractive forces between its molecules and the molecules of the liquid. A dissolving polar gas may partially react with the liquid to form additional compounds. If the dissolving gas is extremely reactive (HCl, NH_3 dissolving in water) several thousand volumes may dissolve in 100 volumes of liquid at room temperature. The amount of gas dissolved in a liquid decreases very rapidly with increase in temperature. The high solubility of hydrocyanic acid in water is a serious disadvantage in fumigation practice, in that high toxic concentrations accumulate in the treated commodities. Furthermore, hydrocyanic acid is relatively stable in biological materials at less than pH 7.0 but forms metallic salts above pH 7.0. In contrast to hydrocyanic acid, ethylene chlorobromide, ethylene dibromide, and methyl bromide have relatively slight solubility in water; thus under identical experimental conditions, these slightly water-soluble fumigants will remain in the gas phase longer than a highly water-soluble fumigant like hydrocyanic acid.

It can be observed from the facts presented above that the water content of a food commodity is important in relation to the amount of fumigant sorbed during a fumigation period. Subsequently, this condition usually results in a higher chemical residue if the fumigant that has been used has a relatively high solubility in water.

CHEMICAL RESIDUES IN FUMIGATED COMMODITIES.—The amount and type of residue found in a fumigated commodity (biological material) depends not only on the particular fumigant used but, in many instances, on the degradation products which are formed as a result of the chemical reactions that occur in the material subsequent to fumigation. Some fumigants are very stable and consequently do not react chemically with the biological material that has been fumigated. In such instances the chemical residue determinations involve the analysis of the material for the amount of fumigant remaining in the samples several days after the fumigation period. Under such experimental conditions, no attempt has to be made to establish the chemical residue content of a fumigated commodity by analyzing samples for degradation products.

Some fumigants not only are unstable and decompose when sorbed by a commodity, but also react chemically to form new compounds, degradation and addition products with substances in the fumigated material. In many instances these degradation or addition products are as toxic as the original fumigant and remain in the commodity as a toxic residue.

To illustrate these principles, let us compare the chemical behavior of a few fumigants that are used to treat various food stuffs for insect infestations. In addition to being highly soluble in water, hydrocyanic acid is highly sorbed by food materials during fumigation. Owing to its high vapor pressure, much of the sorbed HCN will be given off to the atmosphere. If the commodity that has been treated with HCN has a pH 7.0 or above, much of the sorbed HCN will be converted to the metallic salts, which are relatively stable compounds and will not evaporate into the atmosphere, and they are just as toxic as the free acid form.

Methyl bromide is another fumigant which has a characteristic chemical behavior when it is sorbed by food stuffs during fumigation. This fumigant is relatively insoluble in water and has a comparatively high vapor pressure. Consequently, much of the sorbed methyl bromide is released to the atmosphere in a few hours after fumigation, but the amount that remains reacts chemically with the constituents of the treated commodity and thus results in an increased bromine residue. In most food materials these bromine residues are chiefly inorganic bromides which are not highly toxic. In food materials having a high fat content such as walnuts and avocados, it is highly probable that a large portion of the bromide residue is in the organic form as a result of the reaction of the bromide ion with the unsaturated or double bonds of the fat fractions.

Ethylene dibromide is a widely used fumigant that is slightly soluble in water and does not have as high a vapor pressure as methyl bromide at ordinary temperatures. Consequently, it has a tendency to remain in the treated commodity longer than methyl bromide. Bromide residues exist in greater amounts in food commodities that have been fumigated with ethylene dibromide as a result

of the reaction of the fumigant with the biological tissues that have been treated.

It can be reasonably assumed that the high retention of bromine by avocados is positively correlated with the high fat and oil content of the fruit. In addition to the formation of inorganic bromides as a result of the decomposition of methyl bromide in the tissue, it is probable that a portion of the bromine ion reacts with the unsaturated fatty acids to form stable organic bromides. Since neither the organic bromides nor the inorganic bromides are volatile constituents, they would remain in the tissue as a permanent bromide residue.

In general, the amount of bromine retained by citrus fruit on fumigation with methyl bromide was proportional to the dosage used in the fumatorium, but exceptions were noted, owing to factors such as fruit maturity, district in which the fruit was grown, and the physical and chemical nature of the peel.

Bromine residues in the pulp after fumigation with methyl bromide show the amount that diffuses through the peel. The thickness of the peel is an important factor in the total bromine residue determined in the vesicles of the pulp, as well as dosage and time of exposure to the fumigant.

The bromine residues reported in table 2 were determined on Fuerte avocados fumigated with ethylene chlorobromide and ethylene dibromide for a 2-hour exposure at 75° F. Fruits fumigated at different times varied considerably in the amount of bromine retained, the amount initially absorbed during fumigation depending largely upon the rind thickness and the degree of maturity of the fruit at the time of treatment. During the first 24 hours after fumigation, the greatest quantity of the total bromine initially absorbed was given off. From then until the end of the seventh day, only a slight decrease in total bromine residue occurred.

Ethylene chlorobromide has a lower specific gravity, boiling point, and molecular weight, but has a higher vapor pressure than ethylene dibromide. These differences in physical properties should indicate that ethylene chlorobromide would evaporate faster from fumigated avocados than ethylene dibromide. The bromine residues reported in table 2 support these views, in that the ethylene chlorobromide that is equivalent to the bromine is much less than similar values of ethylene dibromide in comparable samples fumigated under identical conditions of concentration and exposure.

SUSCEPTIBILITY OF COMMODITIES TO INJURY BY FUMIGATION.—At a given concentration and period of exposure, some commodities can withstand fumigation treatments, while others are seriously injured. In a defined fumigation experiment, the problem, therefore, is to determine the susceptibility or resistance of the commodities to the fumigant in the gaseous state at various dosages and periods of exposure.

The nature of the injuries to treated commodities depends upon the physiological characteristics of the material being fumigated. In the fumigation of citrus and avocado fruits against fruit fly infestations, the type of injury that occurred was manifested by a gradual breakdown of both the oil glands and the surrounding cellular tissues, resulting in deep-pitted spots which ultimately developed into small or relatively large brown areas on

Table 2.—Bromine residue in peel and pulp of avocado fruit after fumigation for 2 hours at 75° F.

After Fumigation	Mg. Bromine/100 Grams Fresh Weight After Fumigation					
	0.5 Lb./1000 Cu. Ft.			0.75 Lb./1000 Cu. Ft.		
	Peel	Pulp	Whole Fruit	Peel	Pulp	Whole Fruit
	Ethylene Chlorobromide (ECB)					
1 hour	3.57	1.72	1.94	4.34	2.54	2.75
24 hours	2.82	1.26	1.45	3.42	2.12	2.27
48 hours	2.59	1.18	1.34	3.00	1.74	1.89
7 days	2.36	1.10	1.25	2.80	1.65	1.79
	Ethylene Dibromide (EDB)					
4 hours	9.06	3.25	3.95	11.40	4.15	5.02
28 hours	4.24	2.94	3.10	5.64	3.70	3.94
52 hours	4.13	2.93	3.07	5.14	3.88	3.93
9 days	4.10	2.80	3.05	4.80	3.70	3.90

the fruit surface. The injury produced by the various fumigants was slow in developing but usually appeared within 10 days to 2 weeks after fumigation. The degree of injury was greatly dependent upon the storage temperature after fumigation. Those fruits stored at 45° F. did not develop injury as quickly as those stored at room temperatures. However, some fruit samples that were only slightly injured after 2 weeks of storage at room temperature developed severe discoloration and breakdown after 5 to 6 weeks.

In these studies, the degree of injury was classified as "slight," "moderate," or "severe," the rating depending upon the amount of injury evident on the fruit surfaces. Occasionally, a fruit was classified as "slightly injured." Such injury may have been caused by handling of fruit during picking or in transferring to field boxes. These injuries, which were not apparent at the time of fumigation, may have been accelerated by the fumigant. A fruit damaged by pitting or discoloration up to one-fourth, one-half, or more than one-half of its total surface area was classified, respectively, as slightly, moderately, or severely injured. Segregation of the injured fruits into these three classes was a practical way of correlating the degree of injury with the concentration of the fumigant used in the fumatorium. The comparative effects of the various fumigants on the fruits can be estimated as percentages of total number of fruits slightly, moderately, or severely injured. Obviously, fruit containing any pitting or discoloration could not be shipped to the fresh-fruit market.

All fruit samples were tasted for off-flavor shortly after fumigation and intermittently during the storage period by a taste panel consisting of a number of laboratory workers. In some of the fumigation experiments, the off-flavor did not develop for 10 to 14 days after treatment. In most instances where distinct off-flavor occurred, the fumigant used produced a high incidence of pitting and browning on the surface of the fruit.

In these investigations it has been shown that ethylene dibromide caused severe injury to citrus fruits at a 2-pound dosage. The effects of methyl bromide at this same concentration bordered between injurious and noninjurious, their nature depending upon the condition of the fruit. The uncertainty of the results at a schedule of 2 pounds of methyl bromide for 2 hours suggests that this

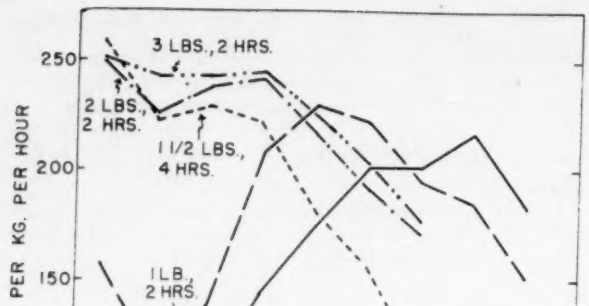


FIG. 10.—Relative respiration rates of preclimacteric avocado fruits stored at 68° F. after fumigation at 70° F. with various dosages of ethylene dibromide per 1000 cu. ft. for different periods. (Eaks & Sinclair, 1955).

concentration is too high to use as a sterilization treatment for citrus fruits.

During our fumigation studies considerable evidence has accumulated to show that the shelf-life of many food commodities has been materially reduced by exposure to an excessive concentration of a fumigant which readily affected the metabolic rate but produced no apparent physical injury to the external surface of the treated commodity. This can be well illustrated by the data of Eaks & Sinclair (1955), in figure 10, which show the comparative effects of different concentrations of ethylene dibromide on the respiration rates of avocado fruits subsequent to the treatment. Avocados fumigated with 1 pound for a 2-hour exposure respired at an initial rate approximately 50% higher than untreated fruit, but went through a normal climacteric rise to a peak, after 5 days, of nearly the same magnitude as the peak reached by the control fruit after 8 days. At the higher dosages the fruits produced essentially the same initial amount of carbon dioxide and showed no climacteric rise in carbon dioxide production. In all fumigation experiments an acceleration occurred in the rate of fruit respiration, and, in general, the higher the rate of CO_2 production was, the faster was the rate of fruit deterioration. These data seem to indicate that the initial rate of respiration of fumigated avocado fruit, and the change in rate during storage, is highly correlated with the subsequent response of the fruit during storage, as well as with the shelf-life of the fruit in commercial channels.

REFERENCES CITED

Bartholomew, E. T., W. B. Sinclair, and D. L. Lindgren. 1942. Measurements on hydrocyanic acid absorbed by citrus tissues during fumigation. Hilgardia **14**: 373–409.

Bhambhani, H. J., and R. E. Blackith. 1958. Responses of pests to fumigation. VII.—The relation between fumigation techniques, mortality, and the amount of hydrogen cyanide sorbed by *Calandra* spp. Bull. Ent. Res. **49**: 165–75.

Eaks, Irving L., and W. B. Sinclair. 1955. Respiratory response of avocado fruits to fumigation effective against the eggs and larvae of fruit flies. Jour. Econ. Ent. **48**: 369–72.

Roark, R. C., and O. A. Nelson. 1929. Maximum weights of various fumigants which can exist in vapor form in a 1000 cubic foot fumigating chamber. Jour. Econ. Ent. **22**: 381–7.

Shepard, H. H., D. L. Lindgren, and E. L. Thomas. 1937. The relative toxicity of insect fumigants. Minnesota Agr. Expt. Sta. Tech. Bull. **120.**

Sun, Yun-Pei. 1947. An analysis of some important factors affecting the results of fumigation tests on insects. Minnesota Agric. Expt. Sta. Tech. Bull. **177.**

Studies on Hydrogen Phosphide as a Fumigant[1]

D. L. LINDGREN, L. E. VINCENT, and R. G. STRONG, *University of California Citrus Experiment Station, Riverside*

ABSTRACT

Hydrogen phosphide evolved from aluminum phosphide has been used for the fumigation of stored grain in Germany for many years. Experimental data indicate that hydrogen phosphide (Phostoxin) is effective as a fumigant against *Tribolium confusum* Duv., *Sitophilus granarius* (L.), *S. oryza* (L.), *Trogoderma granarium* Everts, *Rhyzopertha dominica* (F.), and *Oryzaephilus surinamensis* (L.), and readily penetrates flour or wheat. No effect on seed germination has been observed, and preliminary results indicate no effect on baking quality.

Hydrogen phosphide (H_3P), a colorless, poisonous, spontaneously inflammable gas, has been experimented with as a fumigant for stored-product insects for many years. Recently a method for the evolution of this gas has been developed, which, according to the manufacturer[2] has made it relatively safe to handle. At the present time this product is being used commercially in several wheat-exporting foreign countries, but has not been introduced into the United States.

Heseltine & Thompson (1957), of Great Britain, carried on a series of experiments with aluminum phosphide tablets to assess the efficacy and safety of the product. They concluded: "(1) It should be possible to obtain good results using Phostoxin for the fumigation of grain in the following types of storage: (a) deep silo bins; (b) farm bins; and (c) sacks. (2) There should be no danger to

[1] Accepted for publication September 29, 1958. Partial cost of publication of this paper was met by Hollywood Termite Control Co., Inc., Los Angeles, California.

[2] Phostoxin; manufactured by Degesch-Deutsch Gesellshaft für Schädlingsbekampfung, Germany. American supplier: Hollywood Termite Control Co.

health caused by its use provided reasonable precautions are taken, and there should be no damage to food grain treated with it. (3) Its main advantage would seem to be in the ease of application by personnel with little special skill."

The proprietary compound is manufactured as a highly compressed tablet composed of ammonium carbamate and aluminum phosphide, which on exposure to moisture decomposes to hydrogen phosphide, aluminum hydroxide, ammonia, and carbon dioxide. This decomposition is slow and dependent on the moisture content of the atmosphere and on the temperature. Each tablet weighs 3 grams and upon decomposition produces 1 gram of hydrogen phosphide. This type of tablet was used in the preliminary investigations of hydrogen phosphide reported in this paper.

A series of experiments was conducted to determine the toxicity of hydrogen phosphide to the adults of *Tribolium confusum* Duv., *Sitophilus granarius* (L.), *S. oryza* (L.), *Trogoderma granarium* Everts, *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), and some of the preadult stages under space conditions. The fumigation chambers used were of 28-liter volume without circulation. The insects in cages were placed approximately half-way between the top and bottom of the chamber on $\frac{1}{2}$-inch hardware cloth. The dosages were obtained by cutting the tablet to various sizes ($\frac{1}{16}$, $\frac{1}{8}$, $\frac{1}{4}$, etc.) and weighing these to the closest 0.01 gram and then placing them on a small piece of paper toweling in the fumigation chamber. The exposure was measured from the time the Phostoxin was placed in the chamber and closed. No attempt was made to determine the length of time taken for gas evolution, nor to measure the actual concentration in the chamber. The fumigation chambers were placed in a temperature-controlled room for the exposure period. After fumigation the treated insects along with controls were held at 80° F. and 60% relative humidity until counts were made, which was usually 10 to 14 days after treatment.

Results of these experiments are given in table 1. The larval, pupal, and adult stages of *Tribolium confusum* were all killed at the various dosages, temperatures, and exposure periods tested. Complete kill of the egg stage was obtained with exposures of 48 and 72 hours. The egg, larval, and adult stages of *Sitophilus granarius* were all killed at the various dosages, temperatures, and exposure periods tested. Complete kill of *S. granarius* pupae was not obtained at any of the various dosages, temperatures, and exposure periods. The pupae of *Trogoderma granarium* were killed at all of the various dosages, temperatures, and exposure periods tested, while there was some survival of eggs and larvae at 50° F. Adults of *Rhyzopertha dominica* and *Oryzaephilus surinamensis* were all killed at the various dosages, temperatures, and exposure periods.

The results of fumigating *Sitophilus oryza* egg, larval, pupal, and adult stages were similar to those obtained for *S. granarius*, the pupal stage being the most resistant.

A second series of experiments was conducted to determine the ability of hydrogen phosphide to penetrate through a column of wheat or flour. For experiments measuring the penetration through flour, metal cylinders were used. These cylinders were sealed at the bottom, 4 inches in diameter and 36 inches long. A small amount of flour was placed in the bottom of the cylinder and a known weight of Phostoxin was added. Adult *Tribolium confusum* in probes were placed at intervals of 9, 18, 27, and 36 inches from the bottom as the tube was filled with flour. After the exposure period, the probes containing the test insects were removed from the flour, and the treated insects along with the controls were placed in salve tins with a small amount of food and held at 80° F. and 60% relative humidity until checked for mortality. Complete kill of adult *T. confusum* was obtained in all tests, as shown in table 2.

Further penetration tests were conducted to determine the penetration of hydrogen phosphide through a 6-foot column of wheat in a metal cylinder 8 inches in diameter. Adults of *Tribolium confusum* and *Sitophilus granarius* were placed in probes which were inserted at intervals of 1, 2, 4, and 5 feet from the point of evolution of the gas. A known weight of Phostoxin was placed either at the top or at the bottom of the cylinder. Results are shown in table 2. Complete kill of *T. confusum* adults was obtained at the various dosages, temperatures, and exposure periods tested, while adults of *S. granarius* survived at the lowest dosage tested at 50° F. with an exposure of 72 hours at the greatest distance, below or above, from the point of evolution of the gas.

Table 1.—Results of fumigation tests[a] with hydrogen phosphide on various stages of *Tribolium confusum*, *Sitophilus granarius*, *S. oryza*, *Trogoderma granarium*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis* at 50° and 70° F.

Insect	Dosage Applied (Mg./L.)	Exposure (Hours)	Temperature, (° F.)	Per Cent Kill			
				Egg	Larva	Pupa	Adult
T. confusum	4.8	48	50	100	100	100	100
	4.6	24	70	97.0	100	100	100
	4.4	48	70	100	100	100	100
	4.7	72	70	100	100	100	100
S. granarius	4.5	72	50	100	100	79.8	100
	9.2	72	50	100	100	86.6	100
	4.5	96	50	100	100	88.9	100
	8.9	96	50	100	100	96.5	100
	4.3	72	70	100	100	99.3	100
	9.1	72	70	100	100	99.7	100
	4.3	96	70	100	100	97.4	100
	8.5	96	70	100	100	98.3	100
T. granarium	4.6	72	50	94.0	94.7	100	—
	8.8	72	50	97.5	96.6	100	—
	4.6	96	50	100	99.0	100	—
	8.5	96	50	100	100	100	—
	4.6	72	70	100	100	100	—
	9.2	72	70	100	100	100	—
	4.5	96	70	100	100	100	—
	9.0	96	70	100	100	100	—
R. dominica	4.9	72	50	—	—	—	100
	4.7	96	50	—	—	—	100
	4.5	72	70	—	—	—	100
	4.4	96	70	—	—	—	100
O. surinamensis	4.9	72	50	—	—	—	100
	4.7	96	50	—	—	—	100
	4.5	72	70	—	—	—	100
	4.4	96	70	—	—	—	100
S. oryza	4.6	72	50	100	100	93.9	100
	9.2	72	50	100	100	95.6	100
	4.8	72	70	100	100	99.2	100
	9.2	72	70	100	100	99.9	100
	4.7	96	50	100	100	96.2	100
	9.4	96	50	100	100	97.0	100
	4.9	96	70	100	100	100	100
	9.8	96	70	100	100	100	100

[a] Averages of three or more replications.

Table 2.—Results of fumigations measuring the penetration properties of hydrogen phosphide in bleached flour and wheat.

Dosage (Mg./L.)	Temperature (° F.)	Exposure (Hours)	Gas Generated in Cylinder	Distance of Insects from Point of Gas Evolution (Inches)	Per Cent Kill of Adults	
					T. confusum	*S. granarius*
			Flour (3-ft. tube)			
9.2	70	8	Bottom	9	100	—
				18	100	—
				27	100	—
				36	100	—
10.1	70	24	Bottom	9	100	—
				18	100	—
				27	100	—
				36	100	—
			Wheat (6-ft. tube)			
2.7	50	72	Top	12	100	100
				24	100	100
				48	100	100
				60	100	97.9
2.4	50	72	Bottom	12	100	100
				24	100	100
				48	100	100
				60	100	98.0
5.1	50	72	Top	12	100	100
				24	100	100
				48	100	100
				60	100	100
6.0	50	72	Bottom	12	100	100
				24	100	100
				48	100	100
				60	100	100
2.3	70	72	Top	12	100	100
				24	100	100
				48	100	100
				60	100	100
2.6	70	72	Bottom	12	100	100
				24	100	100
				48	100	100
				60	100	100
4.7	70	72	Top	12	100	100
				24	100	100
				48	100	100
				60	100	100
4.9	70	72	Bottom	12	100	100
				24	100	100
				48	100	100
				60	100	100

Table 4.—Results of germination of wheat seed fumigated with hydrogen phosphide.

Dosage (Mg./L.)	Exposure (Hours)	Temperature (° F.)	Per Cent	
			Moisture Content of Grain	Germination
2.6	72	50	15	95.5
5.6	72	50	15	98.0
2.6	96	50	15	96.0
4.4	96	50	15	95.0
2.9	96	60	10	96.0
2.5	72	70	15	95.5
4.8	72	70	15	94.0
2.3	96	70	15	93.0
5.1	96	70	15	90.0
8.7	24	70	10	95.2
17.9	24	70	10	96.6
5.2	48	70	15	96.0
3.9	48	70	15	96.0
7.7	48	70	15	96.4
Control (average)	—	50–70	10–15	96.0

In a third series of experiments, sacked wheat in loads of 2 to 4 tons under tarpaulins was fumigated with hydrogen phosphide. Test insects used were adults of *Tribolium confusum*, *Sitophilus granarius*, *S. oryza*, and *Rhyzopertha dominica*, and pre-adult stages of *S. granarius*. The tarpaulin consisted of 0.003-inch polyethylene sandbagged at the edges. The sacked grain was on pallets which were on a concrete floor. The Phostoxin tablets were placed singly in open containers in various locations under the tarpaulin. The temperature of the wheat was measured by a recording thermometer. Adult insects were in probes which were inserted at various locations inside the sacks, while the pre-adult stages were in wheat in small cotton bags placed at various locations under the tarpaulin. Following fumigation the treated insects along with the controls were held in salve tins at 80° F. and 60% relative humidity for 10 days or longer, while the pre-adult stages were kept until adult emergence was complete. As many as 700 adults emerged from the control samples. Results are given in table 3. All tests gave 100% kill of the adults of *Sitophilus oryza*, *S. granarius*, *Rhyzopertha dominica*, and *Tribolium confusum*. Larvae of *S. granarius* survived the lowest dosage and exposure tested. A complete kill of pupae of *S. granarius* was obtained with a dosage of six tablets per ton and an exposure of 72 hours, with a grain

Table 3.—Results of fumigation of various insects in sacked wheat with hydrogen phosphide.

Dosage Applied (Tablets/Ton)	Exposure (Hours)	Sack Temperature (° F.)	Per Cent Kill of Adults								Per Cent Kill of *S. granarius*		
			S. oryza		*T. confusum*		*R. dominica*		*S. granarius*				
			Space	In Sack	Space	In Sack	Space	In Sack	Space	In Sack	Eggs	Larvae	Pupae
2	72	64–66	—	—	100	100	—	—	100	100	100	99.8	77.1
2	96	55–60	100	100	100	100	100	100	100	100	100	100	88.8
2	120	54–56	—	—	100	100	—	—	100	100	100	100	95.0
4	72	64–68	—	—	100	100	—	—	100	100	100	100	93.5
4	96	55–60	100	100	100	100	100	100	100	100	100	100	95.7
4	120	50–54	—	—	100	100	—	—	100	100	100	100	97.3
6	72	68–72	—	—	100	100	—	—	100	100	100	100	100
6	96	56–62	100	100	100	100	100	100	100	100	100	100	96.7
6	96	70–78	—	—	100	100	—	—	100	100	100	100	100
6	120	52–56	—	—	100	100	—	—	100	100	100	100	99.1
6	144	52–58	—	—	100	100	—	—	100	100	100	100	100

temperature of 68° to 72° F., while 99.1% kill was obtained with a dosage of six tablets per ton and an exposure of 120 hours and a grain temperature of 52° to 56° F.

Wheat seed was fumigated with hydrogen phosphide to determine the effect on germination. Results are given in table 4. Under the conditions of these tests, no effect on germination was observed.

A single preliminary experiment was conducted in which wheat was fumigated with hydrogen phosphide at a dosage of 16.9 mg./liter. This wheat was milled into whole wheat and white flours. Loaves of whole wheat and white bread were baked and submitted to a taste panel for flavor and odor evaluation, conducted by Dr. W. H. Ewart. No significant differences were observed when compared with bread baked from unfumigated wheat.

REFERENCE CITED

Heseltine, H. K., and R. H. Thompson. 1957. Use of aluminum phosphide tablets for the fumigation of grain. Milling. Part 1, Vol. CXXIX—No. 24, Dec. 14, 1957, pp. 676–77; Part 2, Vol. CXXIX—No. 25, Dec. 21, 1957, pp. 730–32, 52; Part 3, Vol. CXXIX—No. 26, Dec. 28, 1957, pp. 774–75, 78, 83.

Evaluation of Four Insecticides for the Control of Six-Spotted Leafhoppers on Lettuce[1]

R. G. STRONG[2] and W. A. RAWLINS, *Cornell University, Ithaca, N. Y.*

ABSTRACT

Techniques which employed a combination of field and laboratory methods were used to evaluate four insecticides—DDT, demeton, malathion, and parathion—for the control of six-spotted leafhoppers, *Macrosteles fascifrons* ((Stål). With equivalent dosages of toxicant per acre, malathion was as effective at 6 hours as parathion at 12, demeton at 24, and DDT at 48 hours after exposure of leafhoppers to freshly sprayed lettuce plants. Treatments were ineffective in controlling leafhoppers 3 days after application of sprays.

Satisfactory methods are not available for sampling populations of six-spotted leafhoppers, *Macrosteles fascifrons* (Stål), in lettuce growing on New York muckland. The leafhopper, however, is the only known vector of the eastern strain of the virus, *Chlorogenus callistephi* var. *vulgaris* Holmes, which causes lettuce-yellows disease. So the incidence of lettuce yellows has become the standard measurement for the evaluation of insecticides applied to lettuce in the field for leafhopper control. Obviously only an indication of leafhopper mortality due to insecticide sprays can be gained from such an indirect measure of toxicity.

The need for a better method of evaluating insecticide sprays for the control of six-spotted leafhoppers on lettuce led to the use of techniques which employed a combination of field and laboratory methods. This report gives results from time-mortality tests in the laboratory with leafhoppers on lettuce which was sprayed in the field. The technique proved to be so simple and efficient that it was tried later to select minimum dosages required of several insecticides for six-spotted leafhopper control in the field.

MATERIALS AND METHODS.—Dosages of insecticides are given in weight of actual toxicant applied per acre. The amounts of toxicant per gallon in the formulations used were as follows: DDT—2 pounds, demeton—2 pounds, malathion—5 pounds, and parathion—2 pounds.

A low-pressure sprayer mounted on a garden-type tractor delivered 100 gallons of spray per acre through two *TeeJet* 8002 nozzles per row at a speed of 2 m.p.h. and a pressure of 40 p.s.i. Five 15-inch rows were covered in each swath, but test plants were taken from the three center rows.

Cylinders open at both ends were made from waste 1-quart oil cans to remove plants from the field after spraying. The cylinders were placed over lettuce and pushed into the muck soil. Plants were taken intact without disturbing the spray deposit by carefully removing the cylinders with soil. These were hauled to the laboratory.

Leafhoppers were confined to individual lettuce plants in cages 6 inches high and $3\frac{3}{4}$ inches in diameter which fit snugly inside the rims of oil-can cylinders. Cage frames made from cellulose acetate were covered with nylon tulle containing 24×34 threads per square inch. Air circulated freely over the plants and preliminary tests indicated no deleterious effects from insecticide fumes inside cages. Test plants were held for insect mortality observations in insectaries similar to those described by Strong & Rawlins (1956). Test insects were reared on barley in insectary cultures.

TIME-MORTALITY TESTS SUPPLEMENTING FIELD EXPERIMENTS.—As mentioned previously, the only criterion available for evaluating insecticides for six-spotted leafhopper control in New York State lettuce fields is the incidence of lettuce yellows. Thus, obtaining specific information on the toxicity to leafhoppers of sprays applied in the field was an urgent task. Time-mortality tests were conducted to supplement results (to be reported later) from field experiments with insecticides for the prevention of virus transmission.

Experiment 1.—The four insecticidal treatments listed in table 1 were compared for six-spotted leafhopper control. Major consideration was given to the immediate action and residual persistence of insecticides in the field as measured by leafhopper mortality. Treatments were replicated three times in a split plot design. Whole plot

[1] Accepted for publication July 1, 1958.

[2] Present address: Department of Entomology, University of California, Citrus Experiment Station, Riverside, California.

Table 1.—Mean percentage (cumulative) mortality of six-spotted leafhoppers placed on lettuce 2 hours and 3 days after application of emulsion concentrate sprays.

Insecticide	Toxicant per Acre (Oz.)	Time After Spraying: 2 Hours — Per Cent Mortality (Hours After Caging Insects) 12	36	72	3 Days — 72
DDT	8	12	44	57	4
Demeton	3	17	58	81	4
Malathion	20	99	100	100	6
Parathion	4	57	94	99	7
Unsprayed	—	6	12	18	3

treatments were the insecticide sprays and the split plot treatments were the periods from spraying to caging leafhoppers on lettuce.

Sprays were applied at weekly intervals after lettuce was up-to-a-stand. Following the third spray application, 10 plants were taken at random from each field plot. Ten leafhoppers per plant were caged on lettuce within 2 hours after spraying. Mortality records were made at 12, 36, and 72 hours. Three days after spraying the procedure was repeated except that mortalities were recorded only once, 72 hours after caging insects.

Differences between insecticide sprays are readily evident for the period immediately after spraying from the results presented in table 1. It is also apparent that insecticides were not effective in controlling leafhoppers 3 days after application of sprays.

Experiment 2.—Spray treatments were replicated six times in a split plot design. Whole plot treatments were the insecticide sprays given in table 2, and split plot treatments were the periods from spraying to caging leafhoppers on lettuce.

Lettuce was sprayed at weekly intervals, beginning with seedling emergence from the soil. Following the third spray application, 10 plants were taken from the field for each treatment replicate. Ten leafhoppers per plant were caged on lettuce 1 hour after sprays were applied. Mortality observations were made at various intervals as shown in table 2. Three days after spraying the procedure was repeated except that mortalities were recorded only once, 64 hours after caging insects on plants.

Table 2.—Mean percentage (cumulative) mortality of six-spotted leafhoppers placed on lettuce 1 hour and 3 days after application of emulsion concentrate sprays.

Insecticide	Toxicant per Acre (Oz.)	Time After Spraying: 1 Hour — Per Cent Mortality (Hours After Caging Insects) 4	16	28	40	64	3 Days — 64
DDT	12	0	42	57	72	83	13
Demeton	6	21	75	97	99	100	32
Malathion	20	93	100	100	100	100	10
Parathion	6	55	98	100	100	100	21
Unsprayed	—	0	0	1	1	8	10

Table 3.—Comparison of various dosage levels of four insecticides applied to lettuce as emulsion concentrate sprays for six-spotted leafhopper control.

Insecticide	Toxicant per Acre (Oz.)	Per Cent Mortality (Cumulative) Hours After Caging Insects: 1.5	3	6	12	24	48
DDT	8	—	—	6	38	69	78
	16	—	—	6	65	88	94
	24	—	—	24	74	89	97
	32	—	—	30	81	92	97
	Unsprayed	—	—	0	0	1	5
Demeton	4	—	0	17	47	73	—
	6	—	1	28	62	85	—
	8	—	1	50	73	88	—
	16	—	9	71	91	98	—
	Unsprayed	—	0	0	0	4	—
Malathion	10	17	58	94	99	100	—
	15	25	75	97	100	100	—
	20	31	74	97	100	100	—
	40	36	61	98	100	100	—
	Unsprayed	0	0	0	1	3	—
Parathion	4	—	8	49	85	95	—
	6	—	22	67	91	99	—
	8	—	33	79	94	99	—
	16	—	41	86	95	100	—
	Unsprayed	—	0	0	1	3	—

A summary of insect mortalities recorded at various observation periods following plant exposure to leafhoppers is given in table 2. Speed of kill was in the order of malathion > parathion > demeton > DDT, although malathion, parathion, and demeton sprays were equally effective at the end of 28 hours. Little benefit was gained from insecticides 3 days after spraying.

Comparison of Dosage Levels.—Four dosage levels for each of four insecticides were evaluated by the methods used in time-mortality studies supplementing field experiments. Each series of insecticide dosage levels constituted a separate experiment replicated six times in a randomized complete block design. Five lettuce plants were used for each treatment replicate. Insecticidal treatments for all experiments are given in table 3.

Seedling lettuce plants were transplanted into muck soil contained in 1-quart oil-can cylinders. When plants reached a height of approximately $1\frac{1}{2}$ inches, about blocking size, they were sprayed in a manner comparable to field application of insecticides. After spraying, plants were taken to the laboratory where 10 leafhoppers were caged over each. Forty to 90 minutes elapsed between spraying and caging of leafhoppers.

Mortalities were recorded at geometric intervals, beginning 45 minutes after caging leaf-hoppers. However, the summary of results given in table 3 begins with the minimum effective interval and ends with the final observation for each insecticide.

Discussion of Results and Conclusions.—Results presented in tables 1 and 2 from time-mortality studies indicate the relative speed at which leafhoppers are killed by four insecticide sprays. At the dosages applied, malathion appeared to be the most effective insecticide, followed closely by parathion, for the control of six-

spotted leafhoppers. Lack of effective control 3 days after spraying, however, is an important point to consider. Since none of the sprays were very effective for as long as 3 days, added prominence is given to the speed of toxic action. Leafhoppers in lettuce at the time of spraying, or entering fields immediately afterwards, are given more time to feed before they are killed by insecticide sprays with slow toxic action (DDT, for example). If viruliferous insects are present, this could mean an economic differential in the number of plants inoculated with the virus causing lettuce yellows.

From the results presented in table 3, it is apparent that dosages of insecticide required to control six-spotted leafhoppers can be selected efficiently in the laboratory with field-sprayed lettuce. Although strictly valid comparisons cannot be made between the insecticides used in experiments comparing dosage levels, conditions and methods were similar and such comparisons are relevant to expected results in the field. The most equivalent dosages of the four insecticides tested were: malathion—15 ounces; DDT, demeton, and parathion—16 ounces per acre. With these dosages, malathion was as effective at 6 hours as parathion at 12, demeton at 24, and DDT at 48.

Caging leafhoppers leaves much to be desired since the insects often roost on cages for some time before going to lettuce plants. This suggests that the time-intervals required for insect mortality are not altogether correct. However, it is concluded that data obtained from the use of a combination of laboratory and field techniques permits definite comparisons between treatments for leafhopper control and reduces the need for field methods to measure leafhopper populations in lettuce.

REFERENCE CITED

Strong, R. G., and W. A. Rawlins. 1956. Mass rearing of six-spotted leafhoppers. Jour. Econ. Ent. **50**(1): 76–79.

The Translocation of Radioactive (S^{35}) Bayer 19639 in Pineapple Plants[1]

WALTER CARTER and W. A. GORTNER[2]

ABSTRACT

Radioautographs revealed that the radioactive sulfur in S^{35}-tagged Bayer 19639 (Di-Syston) is translocated from the soil through the entire pineapple plant. Absorption is greatest in the roots and decreases to barely discernible quantity in the green half-developed fruit. Bioassays revealed that only in the region of greatest absorption—the roots—was the concentration of Bayer 19639 high enough to be toxic to mealybugs.

The purpose of this paper is to report the results of tests with radioactive Bayer 19639 (Di-Syston) applied to the soil around the base leaves of pineapple plants to determine the degree of absorption and translocation in plant tissues and the persistence of the translocated materials. The experiment had its genesis in the results of a preliminary test of the effect of Bayer 19639 applied in a similar manner for the control of mealybugs, *Pseudococcus brevipes* (Ckl.), on the under-ground portions of the plants. The results of this test were most promising, as the summarized data shown in table 1 will indicate. In this test, the formulation used was a 2½% granular material applied at the rate of 100 pounds per acre.

Table 1.—Live mealybugs found on the underground parts of pineapple plants 2 and 4 months after treatment with Bayer 19639.

	After 2 Months		After 4 Months	
	Check	Treated	Check	Treated
No. of plants sampled	22	46	104	106
No. infested	12	5	75	7
Average no. of mealybugs on infested plants	8	4	60	8

METHODS.—The radioactive (S^{35}) Bayer 19639 which was furnished through the cooperation of Chemagro Corporation was applied as a 1/500 dilution of a 20% emulsifiable concentrate at the rate of 125 c. c. of the diluted material per plant. In each case the liquid was poured onto the soil around the base of the plant. Four separate lots of plants were treated. One was a series of young plants just starting to grow in 1-gallon cans from slips which were initially infested with mealybugs. The second was a series of vegetative field-grown plants 1 year old. The third and fourth series were plants at the differentiation point and in the early fruiting stage, respectively. Bioassays were performed on aliquots of the leaf and stump tissue of the plants after each sampling. Gravid female mealybugs were caged on leaf tissue and the development of colonies observed. Stump tissue and root masses were kept in glass jars and the mealybugs released onto them.

The Bayer 19639 was tagged with sulfur-35 in the side chain, the two sulfur atoms directly attached to the central phosphorus atom being non-radioactive. Synthesis was carried out by Dr. Muhlmann in the laboratories of Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany. The specific activity was 4.76 millicuries/gm. at the start of the experiment; the applied Bayer 19639 contributed radioactivity amounting to approximately 272 microcuries per plant.

At various intervals ranging up to 16 weeks after treatment, plants were harvested and the leaves and roots removed. Selected leaves and roots were thoroughly scrubbed to remove contaminating radioactive soil, wiped dry, and retained for use in radioautography. A

[1] Published with the approval of the Director as Technical Paper No. 252 of the Pineapple Research Institute of Hawaii. Accepted for publication July 7, 1958.

[2] Pineapple Research Institute, Honolulu, Hawaii.

FIG. 1.—Radioautograph of pineapple tissue from small plant treated with Bayer 19639. Harvested after 8 weeks; radioautograph time, 45 days.

thin vertical section of the stem and the fruit (when the latter was present) was also used, together with thin sections of the plant across various parts of the stem and fruit. The plant tissues were placed between filter and blotting papers in a botanical press and dried several days at 70° C.

Radioactivity of the various dried plant parts was measured directly by use of a thin-window Geiger tube over a one-inch hole in a 5-mm. thick sheet of plastic. The indicated radioactivity served as a guide to timing contact with the film in radioautography.

Radioautographs were made by placing a sheet of Kodak No-Screen X-ray film directly on the plant tissue and holding in the botanical press in the dark for 31 to 66 days, after which the film was removed and developed. In each of the four tests, the tissue from the first harvest was on the film for 31 days, and subsequent samples were held for sufficiently longer periods to compensate for radioactive decay (87.1 day half-life for S^{35}). Thus the intensity of the blackening of the film is correlated with the amount of radioactive sulfur in the tissue from the applied Bayer 19639.

RESULTS.—Distribution of the radioactive material in the plant tissues was clearly revealed in the radioautographs. An untreated check plant gave a barely discernible picture on the X-ray film.

Series 1.—In the young plants in series 1, radioactivity was clearly visible throughout the plant harvested 2 weeks after treatment, with a high concentration in the roots and some in the short basal leaves. A moderate concentration was also observed in the stem particularly in the vascular bundles. The long, actively growing leaves showed appreciably less radioactivity.

The plant harvested 8 weeks after treatment showed the maximum radioactivity in this series. The roots showed somewhat less activity than at the 2-week or 4-week harvest periods but the stem and leaves were appreciably more radioactive. A concentration of radioactivity in the vascular bundles was evidenced by a concentration of black dots on the film in the region of the upper stem. This radioautograph from the 8-week harvest is shown in figure 1. The record after 16 weeks shows a definite and uniform reduction in intensity of radioactivity, with the cross sections of the vascular bundles in the upper stem again showing up as black dots on the film.

A plant of crab grass (*Digitaria sanguinalis* (L.) Scop.) grew from seed in one of these pots after the treatment was applied. Absorption and translocation was many times greater in this plant than in the pineapple plant as shown by the 89-hour exposure (fig. 2). Compare this with figure 1, with a 45-day exposure, and with figure 3, with a 56-day exposure on the film.

Series 2.—These plants were one-year-old when treated. Both the 5-week and the 10-week harvest showed a moderate concentration of radioactivity in the roots with a

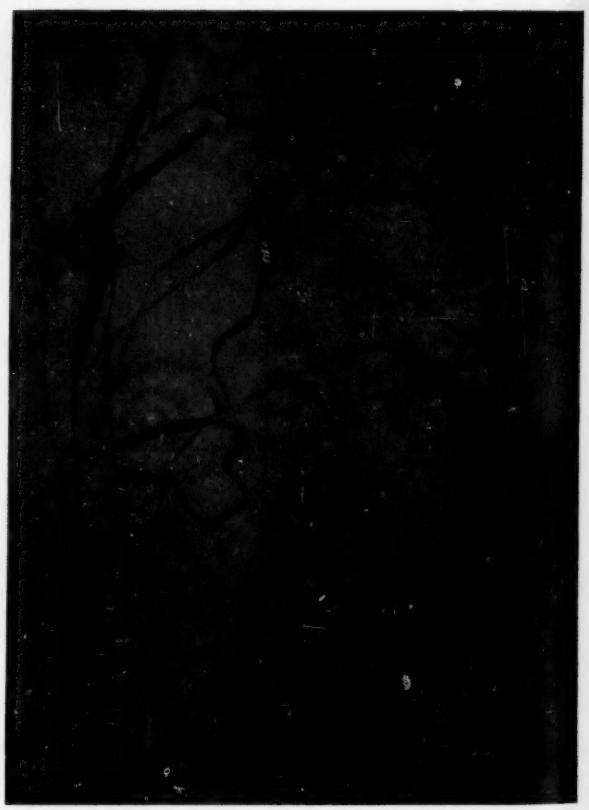


FIG. 2.—Crab grass from the same plant pot as figure 1, but with radioautograph time of 89 hours.

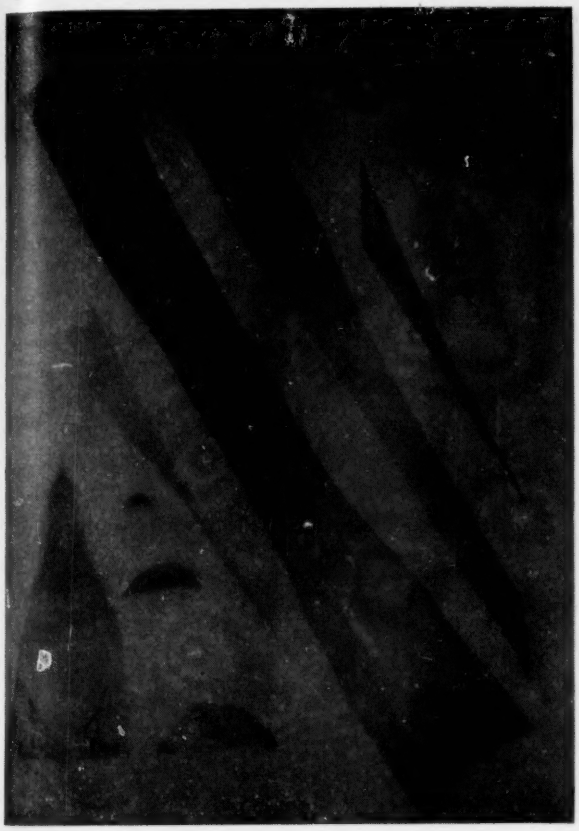


FIG. 3.—Radioautograph at early differentiation stage of pineapple plant treated with Bayer 19639 when mature but vegetative. Note the developing fruit stem clearly but faintly outlined. Harvested after 14½ weeks. Radioautograph time, 56 days.

much lower concentration in the leaf tissue. The radioautographs showed a slightly greater concentration of activity in the leaf bases and in the stem tips. The sample harvested after 14 weeks had differentiated and the emerging peduncle was appreciably more radioactive than the lower portion of the stem, figure 3.

Series 3.—These plants when treated were past the early stages of differentiation. Again the roots showed up as dark areas on the film even in the plant harvested after 4 days. In this sample, weak but noticeable radioactivity was also present in the stump and in the mature leaves, which were clearly outlined on the film. The plant harvested 2 weeks after treatment showed the highest concentration of radioactivity in the basal portion of the older leaves and in the emerging peduncle at the stem tip. A small fruit had developed on the plant harvested nearly 11 weeks after Bayer 19639 treatment. The radioactivity in the core and peduncle was of the same order of magnitude as in the leaf tissue, which was somewhat higher than after 2 weeks.

Series 4.—These plants had small fruits at the time of treatment. Essentially no radioactivity was detected in the plant harvested after 1 week. After 2 weeks, the radioactivity was clearly evident in the mature leaves as well as in a small sucker developing at the base of the peduncle. Very weak radioactivity made the younger leaves and the image of the peduncle for its entire length just visible on the film. The fruit was free from radioactivity at this period.

After 4 weeks, the concentration of radioactivity in the mature leaf was similar, and slightly more radioactivity was evident in the other tissues than at the earlier sample. The developing sucker, the peduncle and the fruit and crown were faint but nevertheless clearly defined on the film. The distribution and intensity of the radioactivity after nearly 11 weeks remained unchanged.

BIOASSAYS.—In order to determine the significance of the radioautographs with respect to insecticidal value of the translocated material, bioassays were performed on tissues comparable to those used to produce the radioautographs. In the case of leaf tissue, gravid female mealybugs were caged on the leaves and subsequent colony development noted. On stump tissue and root masses, mixed colonies were released. The results of these tests showed that detached leaves of treated plants as well as stumps cleaned of roots were not toxic to mealybugs since colonies developed without exception. However, introduced colonies of mealybugs failed to survive on the root masses of plants from the third and fourth series.

The translocated sulfur detected by radioautography may possibly represent movement of a degradation product of the radioactive Bayer 19639. However, the presence of Bayer 19639 in toxic amounts in the roots but not in other parts of the plant as demonstrated by bioassay is in accord with the distribution of radioactivity in the tissues. Furthermore, the distribution of the radioactive sulfur was grossly different from the normal distribution of sulfur in the pineapple plant. The normal pattern is for a relatively uniform distribution throughout the green and etiolated basal leaf tissues, the fruit, and the roots with some 5 to 10 times more in the stem than in the other tissues.

House Fly Control Studies in Chatham County, Georgia[1]

JOHN W. KILPATRICK and H. F. SCHOOF

ABSTRACT

In 1957, residual applications of chlorinated hydrocarbons and organophosphorus compounds and insecticide impregnated cord installations were evaluated in Chatham County, Georgia, for control of house fly populations. EPN:DDT suspensions (25:200 mg./sq. ft.) provided satisfactory fly control for 3 to 7 weeks, an EPN:sugar suspension (25:62.5 mg./sq. ft.) failed to provide control, and a DDT suspension (200 mg./sq. ft.) gave excellent to satisfactory control for 8 weeks. Dow ET-57 (sampled as Dow ET-14) emulsions and suspensions produced acceptable control for 9 to 11 weeks (200 mg./sq. ft.) and for 4 to 5 weeks (100 mg./sq. ft.). A Dipterex:sugar water solution 200:500 mg./sq. ft.) yielded excellent control for 10 weeks at one dairy but was effective for only 2 weeks at a second dairy. Re-treatment of the latter dairy provided 6 weeks of satisfactory control. Emulsions of malathion:DDT:sugar; Guthion:sugar; General Chemical Compound 3707; or suspensions of lindane or methoxychlor failed to give control. Parathion-impregnated cotton cords ($\frac{3}{32}$″ diameter), Diazinon-impregnated cords ($\frac{1}{8}$″ or $\frac{3}{16}$″ diameter) and parathion-Diazinon treated cords ($\frac{3}{32}$″ diameter) provided excellent to satisfactory fly control in dairy barns and a chicken ranch for periods of approximately 9 to 15 weeks.

Investigations for the past 5 years for the control of adult house flies (*Musca domestica* L) have been directed primarily towards the use of organophosphorus compounds (Hansens 1956, Goodwin *et al.* 1956, Kilpatrick & Schoof 1956, 1957, and Schoof & Kilpatrick (1958). With the exception of the impregnated cord treatment, the duration of control was much shorter than that formerly obtained with halogenated hydrocarbon insecticides.

In 1957, house fly control investigations in the Savannah, Georgia, area were continued with selected chlorinated hydrocarbon and organophosphorus compounds as residual spray treatments on dairy farms. Further evaluation of parathion-Diazinon treated cords also was made.

METHODS.—Residual treatments with the insecticides were applied to all potential fly resting surfaces in and around the test dairies at 100 p.s.i. by a power sprayer equipped with flat spray nozzles. Materials tested included EPN and DDT suspensions alone or in combination at dosages of 25 mg. of EPN and/or 200 mg. of DDT/sq.ft.; Dow ET-57 (sampled as Dow ET-14) emulsions and suspensions at dosages of 100 and 200 mg./sq.ft.; Dow ET-57: sugar emulsion at a dosage of 100:250 mg./sq.ft.; Dipterex[2]: sugar water solutions (200:500 mg./sq.ft.); malathion:DDT:sugar (100:100:250 mg./sq.ft.); Guthion:sugar emulsion and General Chemical Compound 3707[3]:sugar emulsion (25:62.5 mg./sq.ft., each); lindane suspension (25 mg./sq.ft.); and a methoxychlor suspension (200 mg./sq.ft.). Commercially prepared wettable powders or emulsifiable concentrates were used to formulate the finished spray.

Cords impregnated with parathion (10% xylene solution, $\frac{3}{32}$″ diameter cords), Diazinon (25% xylene solution, $\frac{1}{8}$″ and $\frac{3}{16}$″ diameter cords) or parathion:Diazinon (10:2.5% xylene solution, $\frac{3}{32}$″ diameter cords), were evaluated in dairies and at a cage type chicken ranch. All cord installations were applied as vertically suspended lengths (18″ to 36″) attached to the ceiling or rafters at a dosage of 30 linear feet of cord/100 sq.ft. of floor area.

The efficacy of all treatments was determined by weekly grill counts of the house flies at each dairy or the chicken ranch. Five counts were made at each of four general locations known to be attractive to high fly populations, the weekly fly index being the average of the four maximum counts, one from each location. Indices of 10 or below were considered as excellent control; those of 20 or below as satisfactory control.

RESULTS.—Immediately after application, fly indices dropped substantially to levels approximating satisfactory control (table 1) at the dairies treated with an EPN:DDT suspension-(25:200 mg./sq.ft.); an EPN:sugar suspension (25:62.5 mg./sq.ft.); and a DDT suspension (200 mg./sq.ft.). The EPN:DDT suspensions provided satisfactory control at dairy "H" for a period of 7 weeks and at dairy "Mo" for 3 weeks. At dairy "Z" with the same treatment, 2 weeks of reduced but unsatisfactory indices were followed by 3 weeks of satisfactory control. The EPN:sugar application at dairy "Ba" failed to provide fly control for even 1 week, although reduced indices were evident for at least 3 weeks following application. The DDT suspension at dairy "W" provided excellent to satisfactory control for a period of 8 weeks.

Dow ET-57 emulsions applied to dairy "Ra" and dairy "Bg" (200 mg./sq.ft.) provided 8 and 9 weeks of excellent fly control (table 2). At dairy "Bk," a Dow ET-57 suspension (200 mg./sq.ft.) provided 8 weeks of excellent control with 1 additional week of satisfactory control. The Dow ET-57:sugar emulsion (100:250 mg./sq.ft.) applied at dairy "Mo" gave 4 weeks of satisfactory control whereas without sugar the same dosage of Dow ET-57 gave 5 weeks of excellent to satisfactory control at dairy "Ba."

A Dipterex:sugar water solution (200:500 mg./sq.ft.) at dairy "Rg" produced an immediate reduction of the fly populations to an excellent control level which was maintained for a period of 10 weeks (table 3). At dairy "S" a similar treatment gave only 2 weeks of satisfactory control but re-treatment 5 weeks later with the same formulation provided 6 weeks of excellent to satisfactory control.

A treatment of malathion:DDT:sugar emulsion (100:100:250 mg./sq.ft.) at one dairy yielded only 2 weeks of satisfactory control. Emulsion:sugar applications of

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia. Accepted for publication July 11, 1958.

[2] Dylox, an agricultural formulation containing 50% of water soluble Bayer L 13/59.

[3] Dimethyl-1,3-di(carbomethoxy)-1-propen-2-yl phosphate.

Table 1.—House fly grill counts in dairy barns near Savannah, Georgia, treated with residual applications of EPN:DDT, EPN:sugar, or DDT suspensions, 1957.

Weeks After Treatment	Dairy "H" EPN:DDT 25:200 Mg./Sq. Ft.	Dairy "Mo" EPN:DDT 25:200 Mg./Sq. Ft.	Dairy "Z" EPN:DDT 25:200 Mg./Sq. Ft.	Dairy "Ba" EPN:Sugar 25:62.5 Mg./Sq. Ft.	Dairy "W" DDT 200 Mg./Sq. Ft.
−2	63	—	78	169	73
−1	200	360	228	324	118
0	352	383	409	482	133
1	13	16	27	31	12
2	11	18	31	30	12
3	10	20	20	34	10
4	16	27	9	62	10
5	20	117	12	99	8
6	16	282	25	136	9
7	15	396	47	176	10
8	24	—	58	—	17
9	32	—	61	—	32
10	28	—	75	—	34
Date treated	5/20	5/23	5/23	5/20	6/10

Guthion or General Chemical Compound 3707 (25:62.5 mg./sq.ft.) and suspensions of lindane (25 mg./sq.ft.) or methoxychlor (200 mg./sq.ft.) failed to reduce fly populations to satisfactory levels even immediately after spraying.

Results of all cord treatments are shown in table 4. The Diazinon-treated cord ($\frac{3}{16}$″ diameter) installed at chicken ranch "R" provided a rapid reduction of the house fly population but 2 weeks were required to reduce indices to a satisfactory level. The latter prevailed for a period of 9 weeks, after which gradually increasing indices were noted. The Diazinon-impregnated cord ($\frac{1}{8}$″ diameter) treatment at dairy "T-1" gave excellent control throughout the entire 15-week observation period. The parathion treated cords ($\frac{3}{32}$″ diameter) installed at dairy "T" reduced fly indices to satisfactory control levels within 3 weeks. Except for post-treatment weeks 7, 8, and 9, this level of abatement persisted for the remainder of the 15-week observation period. Dairy "T" has been treated successfully for 6 consecutive years with parathion-impregnated cords. The cord ($\frac{3}{32}$″ diameter) impregnated with 10% parathion:2.5% Diazinon and installed at dairy "Z" on July 30 provided immediate and effective control for the remainder of the fly season (9 weeks).

Table 2.—House fly grill counts in dairy barns near Savannah, Georgia, treated with residual applications of Dow ET-57.[a] 1957.

Weeks After Treatment	Dairy "Bk" 200 Mg./Sq. Ft.	Dairy "Bg" 200 Mg./Sq. Ft.	Dairy "Ra" 200 Mg./Sq. Ft.	Dairy "Mo" Dow ET-57 Sugar 100:250 Mg./Sq. Ft.	Dairy "Ba" 100 Mg./Sq. Ft.
−2	18	86	—	117	99
−1	60	157	57	282	136
0	96	180	79	396	176
1	3	1	4	12	1
2	2	1	3	16	2
3	3	1	3	10	3
4	6	<1	4	15	6
5	8	0	5	30	16
6	5	2	7	32	37
7	3	4	6	42	73
8	6	4	5	72	132
9	16	7	11	92	185
10	33	13	34	—	—
11	47	16	60	—	—
Date treated	5/27	6/6	5/24	7/8	7/9

[a] Emulsion applications except for suspension at dairy "Bk".

Table 3.—House fly grill counts in dairy barns near Savannah, Georgia, treated with residual applications of Dipterex:sugar at 200:500 mg./sq. ft.

Weeks After Treatment	Dairy "Rg"	Dairy "S"	
−2	132	140	
−1	228	326	Re-treatment
0	319	441	(243)
1	3	7	16
2	4	16	11
3	4	27	7
4	5	73	9
5	5	169	10
6	8	243	19
7	11	—	39
8	9	—	93
9	7	—	154
10	9	—	—
11	28	—	—
12	62	—	—
Date treated	5/13	5/13	6/24

Discussion.—The variable results obtained with formulations of EPN:DDT and with Dipterex may be attributed in part to differences in fly production potential. Presumbably a slow-down in replenishment of the fly population may be the basis for the increase in residual effectiveness apparent during the second treatment of Dipterex at Dairy "S." The contrast in effectiveness between the EPN and DDT applications together with erratic results obtained with the EPN-DDT combinations suggests that the latter may have been due to differences in the relative susceptibility to DDT of the house fly populations at dairies "H", "Mo", and "Z." However,

Table 4.—House fly grill counts in dairy barns and a chicken ranch near Savannah, Georgia, treated with insecticide-impregnated cords installed at the rate of 30 feet of cord per 100 square feet of floor area, 1957.

Weeks After Treatment	Chicken Ranch "R" (3/16″ D. 25% Diazinon)[a]	Dairy "TI" (1/8″ D. 25% Diazinon)[a]	Dairy "Z" (3/32″ D. 10% Parathion: 2.5% Diazinon)[a]	Dairy "T" (3/32″ D. 10% Parathion)[a]
−2	112	20	58	37
−1	337	38	61	95
0	662	48	75	169
1	38	6	4	21
2	29	6	4	35
3	17	4	3	20
4	16	4	3	16
5	17	4	4	13
6	13	6	4	14
7	14	6	4	21
8	15	5	4	28
9	12	6	2	25
10	11	6		16
11	12	7		10
12	32	7		9
13		6		12
14	64	4		13
15	67	3		11
Date treated	5/31	6/18	7/31	5/17

[a] Figures in parentheses represent diameter of cotton cord and strength of impregnating solution.

exposure of flies from all five dairies, treated with DDT and/or EPN, to DDT residues on plywood panels yielded mortalities of similar magnitude and at a level indicative of DDT resistance. Nonetheless, the logical assumption from the results obtained is that the efficacy of the EPN-DDT combinations was due chiefly to DDT.

Dow ET-57 in all dairy treatments gave satisfactory results, both suspensions and emulsions producing comparable control. The addition of sugar to Dow ET-57 formulations did not appear to improve the results obtained. In view of the relatively low mammalian toxicity values of Dow ET-57, the compound has much promise as a residual treatment in fly control.

The Diazinon-treated cord ($\frac{1}{8}$" diameter) produced a level of fly control (15 weeks) superior to the 7 weeks of control achieved in previous studies (Kilpatrick & Schoof 1956) with cord $\frac{3}{32}$" in diameter but closely parallel to those obtained in earlier tests with a $\frac{3}{16}$" diameter cord (Schoof & Kilpatrick 1957).

REFERENCES CITED

Goodwin, W. J., and F. R. Gressette, Jr. 1956. Residual house fly control in dairy and beef barns in South Carolina. Jour. Econ. Ent. **49**(5): 622–4.

Hansens, Elton J. 1956. Control of house flies in dairy barns with special reference to Diazinon. Jour. Econ. Ent. **49**(1): 27–32.

Kilpatrick, John W., and H. F. Schoof. 1956. The use of insecticide-treated cords for housefly control. Publ. Health Repts. **71**(2): 144–9.

Kilpatrick, John W., and H. F. Schoof. 1957. House fly control in dairy barns with residual treatments of organic phosphorus compounds. Jour. Econ. Ent. **50**(1): 36–9.

Schoof, H. F., and John W. Kilpatrick. 1958. Organic phosphorus compounds for the control of resistant house flies in dairy barns. Jour. Econ. Ent. **51**(1): 20–23.

Schoof, H. F., and John W. Kilpatrick. 1957. House fly control with parathion and Diazinon impregnated cords in dairy barns and dining halls. Jour. Econ. Ent. **50**(1): 24–27.

BOOK REVIEW

METHODS OF TESTING CHEMICALS ON INSECTS. VOLUME 1. Edited by Dr. H. H. Shepard. U. S. Department of Agriculture. 8 vo., cloth, 356 pp., illus., Minneapolis, Minn., Burgess Pub. Co., 1958. $5.00.

It is the purpose of this manual to assemble for convenient reference and use methods for studying the various phases of the action of chemicals on insects. This is the first of what is hoped will be a series of three volumes. In this first volume are included methods for the study of fundamental phases (effects of chemicals on the physiology of the insect) and general techniques for applying chemicals to insects. A second volume, now in preparation, will consider special purpose techniques (wood impregnation, soil treatment, repellents, acaricides, etc.) and a discussion of factors known to affect test results (pre-test, exposure, and post-exposure conditions). If and when a third volume appears it should include essential material not previously treated in earlier volumes and will attempt to bring down to date topics previously discussed.

Some idea of the scope and authorship of this work may be gained by enumeration of the respective chapters, as follows: "Surface phenomena in relation to insect cuticle," by W. M. Hoskins, University of California; "Penetration of insect cuticle," by A. G. Richards, University of Minnesota; "Measurement of insect respiration," by R. Craig, University of California; "Electrophysical preparations in the American cockroach," by K. D. Roeder and E. A. Weiant, Tufts College; "Study of the circulatory system in insects," by R. L. Patton, Cornell University; "Radioactive tracer methods," by A. W. Lindquist, U. S. Department of Agriculture; "Resistance studies," by W. V. King, U. S. Department of Agriculture; "Methods of topical application and injection," by R. L. Metcalf, Citrus Experiment Station, Riverside California; "Feeding and drinking methods," by F. W. Fisk, Ohio State University; "Dipping methods," by A. H. McIntosh, Rothamsted Experimental Station; "Precision spraying," by C. Potter and M. J. Way, Rothamsted Experimental Station; "Precision dusting," by J. E. Dewey, Cornell University; "Testing fumigants," by R. T. Cotton, U. S. Department of Agriculture, and "Synergism and antagonism," by N. Turner, Connecticut Experiment Station. Preceding an excellent general index to all the sections, there is also included in single alphabetical sequence a comprehensive list of the literature cited in all the chapters. This comprises 566 titles and possesses much human interest entirely aside from its bibliographical value, since it enumerates by name the various individuals who in recent years have been working on and reporting results secured by means of the various techniques described.

Some years ago the Entomology Subcommittee, Chemical-Biological Coordination Center of the National Research Council, designated Dr. Shepard as editor of its proposed manual of entomological testing methods. It was expected originally that such manual would be published by the Council. Reorganization of the Coordination Center and finally its termination resulted in all rights and responsibilities being relinquished to the editor who then arranged for publication commercially.

On a subject such as this, where advancement in knowledge has become so very rapid that information already in galley proof often becomes obsolete before reaching book form, they are indeed courageous workers who undertake presentation of anything like this down to date comprehensive work on it. Our good friend, the Editor, and his colleagues are indeed to be commended and congratulated, working under such difficulties, on the excellence and practical usefulness of the results of their efforts as thus far presented in this first book of the series.

J. S. WADE

SCIENTIFIC NOTES

Comparisons of Diazinon, Dicapthon, Chlorobenzilate and Kelthane for Control of the Chicken Mite in Hen Houses[1]

J. L. Rodriguez, Jr., and L. A. Riehl, *University of California, Citrus Experiment Station, Riverside*

In southern California the chicken mite, *Dermanyssus gallinae* (DeG.), is a common pest of chickens in hen houses with a floor at ground level and with roosts, dropping boards and box-type nests. In this type of hen house control of the chicken mite with malathion was demonstrated by Raun (1956) with concentrations of 1% in spray or 4% in dust applied at 1 pound per 20 sq. ft. and by Furman & Weinman (1956) with 3% in spray. The importance of this pest offers good reason for tests to develop new pesticides for its control; trials of two organic phosphorus and two chlorinated hydrocarbon compounds are reported here.

Methods.—The tests were conducted in hen houses of frame construction and wire fencing with litter on a floor at ground level and with wooden roosts and box-type nests. The roosts were not attached to a wall and did not have a fixed position in the pen; several separate roosts were used in each pen. Nest boxes arranged in three tiers were supported by a wooden wall and were removable. The number of White Leghorn hens in each pen varied from 250 to 500. Each treatment was used in each of two pens assigned at random; one pen was left untreated as a check.

The spray mixtures of Chlorobenzilate and Diazinon were prepared with the 25% and of Kelthane with the 18.5% emulsifiable liquid concentrate; those of dicapthon (Am. Cyanamid 4124) with the 50% wettable powder. The concentrations given for the treatments represent actual chemical by volume or weight respectively.

Prior to spraying the obvious masses of caked droppings were knocked off the roosts by scraping and the dislodged manure was removed from the house. A hand-pump compressed-air 3-gallon sprayer was used to apply the spray to the roosts, both exterior and interior surfaces of the nest boxes and the wall supporting the nest boxes. Enough spray was applied to wet the structure completely, but care was taken to avoid run-off and the occurrence of wet spots in the litter. Precautions were taken to keep the spray away from feed and water troughs, hens and eggs; the hens were not allowed into the nest boxes until they were dry.

The chicken mite infestation was estimated by counting the mites in each pen at each of six sites each on the roosts and nest boxes. Counts were made each week. At each site the debris that could be dislodged readily from an area of approximately 1 square inch was collected in a shallow pan. The mites that could be seen quickly and easily with the unaided eye were counted through 100 and then listed as 100+; the sample of debris was discarded.

Table 1.—Comparisons of four pesticides applied[a] in spray at various concentrations for control of the chicken mite.

Pesticide and Per Cent Concentration		Mean Number Mites per Count Site					
		Before Treatment	After Treatment (Days)				
			7	14	21	91	105
Untreated	—	100+	100+	100+	100+	100+	100+
Organic phosphorus compounds							
Diazinon	0.25	100+	1.8	0.9	2.7	1.3	12.0
	0.50	100+	3.0	0.0	0.0	0.0	0.0
Dicapthon	0.25	100+	2.0	3.1	5.0	19.1	75.0
	0.50	100+	0.4	0.0	0.0	0.0	0.0
Chlorinated hydrocarbon compounds							
Chlorobenzilate	0.50	100+	100+	100+	100+	—	—
	1.0	100+	100+	1 0+	100+	—	—
	2.0	100+	100+	100+	100+	—	—
Kelthane	0.5	100+	100+	100+	100+	—	—
	1.0	100+	100+	100+	100+	—	—
	2.0	100+	100+	100+	100+	—	—

[a] Applied to roosts and nest box areas of hen houses with litter on a floor at ground level.

Discussion of Results.—Results shown in table 1 indicate that both Diazinon and dicapthon are efficient for control of the chicken mite. Infestations of the chicken mite were not controlled by the residual films of either Chlorobenzilate or Kelthane at the spray concentrations used although mites hit directly by the spray at the time of application were killed. The data of table 1 indicate that the efficiency of Chlorobenzilate and Kelthane against the chicken mite is less than that reported for malathion.

After 21 days counts of the mites were discontinued in the pens treated with Chlorobenzilate and Kelthane. During the interval of 21 to 84 days after treatment the infestation of mites in the pens treated with 0.25% Diazinon or dicapthon remained at the level found at 21 days.

References Cited

Furman, D. P., and C. J. Weinman. 1956. Toxicity of malathion to poultry and their ectoparasites. Jour. Econ. Ent. 49(4): 447–50.

Raun, E. S. 1956. Chicken louse and mite control with malathion formulations. Jour. Econ. Ent. 49(5): 628–9.

[1] Accepted for publication June 9, 1958. The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

Effectiveness of Different Formulations of Insecticides against California Red Scale[1]

A. W. Cressman, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

In the screening of new materials against the California red scale, *Aonidiella aurantii* (Mask.), at Whittier, California, reproducing scales on lemons are treated with wettable-powder sprays in the laboratory and held outdoors until mortality counts are made about 8 weeks later. The survivors continue to reproduce during this interval, and the number of scales of the new generation that become adult gives a measure of the residual activity of the spray deposit (Cressman *et al.* 1957).

Parathion, which is used as a standard of comparison, performs well in both direct and residual action. Some of the materials screened had little effect on mature scales but a pronounced effect on the young emerging after the spraying. For example, Guthion and Trithion sprays were less than one-fourth as effective as parathion on mature scales, but their residual effectiveness was equal to or better than that of parathion.

It is not known how parathion reaches the scale insect beneath its covering. The compound has considerable fumigating action in confined space, and diffusion of vapor from the spray deposit surrounding the covering is a possible means of access. If the vapor pressures of Trithion and Guthion are too low for effective vapor concentrations around the scale body, a carrier to take the toxicants beneath the covering should increase their effectiveness. In order to test this possibility, emulsified kerosene solutions of these compounds and an emulsifiable concentrate of Trithion were evaluated and the results compared with those

[1] Accepted for publication July 7, 1958.

Table 1.—Effectiveness of different formulations of organic phosphorus compounds against mature California red scales.

Material	Formulation	Grams of Toxicant per Liter	Per Cent Mortality
	Series 1		
Trithion	25% WP	0.50	22
	50% EC	.50	67
	Kerosene solution	.16	95
Guthion	25% WP	.50	28
	Kerosene solution	.16	88
Parathion	25% WP	.50	84–95
	Kerosene solution	.16	95
Kerosene	—	—	26
	Series 2		
Hercules AC-528	25% WP	0.50	1
	Kerosene solution	.20	63
		.60	85
Parathion	25% WP	.50	84–95
	Kerosene solution	.20	99+
Kerosene	—	—	16

obtained with wettable-powder formulations. Another series of sprays afforded a similar comparison of an emulsified kerosene solution of Hercules AC-528 (Delnav), which had also shown prolonged residual action from wettable-powder sprays.

Dibutyl phthalate was used as a mutual solvent for Guthion and kerosene. The other compounds were soluble in kerosene at the concentrations used. The kerosene solutions were made emulsifiable with 0.5% of polyoxyethylene sorbitol hexaoleate (Atlox 1086), and the Trithion emulsifiable concentrate was made with xylene and polyoxyethylene sorbitol esters of mixed fatty and resin acids (Atlox 1256).

The strengths at which the sprays were applied and results are shown in table 1.

With Trithion the increasing order of effectiveness was wettable powder, emulsifiable concentrate, and kerosene solution. The other compounds also showed a marked increase in toxicity when applied in the kerosene solutions, although Hercules AC-528 was less toxic than parathion in a similar formulation.

Solutions of toxicants in oils are not new in scale-insect control. Several years ago there were extensive investigations of rotenone and other toxicants in oil (Boyce *et al.* 1940, Ebeling 1941, Cressman 1943). The organic-phosphorus compounds broaden the range of materials that may be used in this way. The results indicate that if deposits from wettable-powder sprays show prolonged residual action on young of armored scales, solutions in liquid carriers should be tested against the adult scales. Trithion and also ethion, another material that showed good residual activity in laboratory tests, are now available in formulations prepared especially for use with kerosene or oil.

Early field work with parathion showed that kerosene and oil solutions were more phytotoxic than the wettable powders. This would probably hold true for other compounds, but might be compensated for by a decrease in the amount required for control.

References Cited

Boyce, A. M., J. F. Kagy, G. L. McCall, and J. P. LaDue. 1940. Black scale control. Summary of studies with low dosages of oil with rotenone-bearing materials. California Citrograph **25**(10): 314, 342–4.

Cressman, A. W. 1943. Effectiveness against the California red scale of cube resins and nicotine in petroleum spray oil. Jour. Agric. Res. **67**(1): 17–26.

Cressman, A. W., Bessie M. Broadbent, and Francis Munger. 1957. Screening tests of some organic insecticides against the California red scale. U. S. Dept. Agric. **ARS-33-40.**

Ebeling, Walter. 1941. Materials added to oil spray to increase its effectiveness in citrus pest control. Jour. Econ. Ent. **34**(6): 829–37.

Heat Treatment for Elimination of the European Chafer[1]

Everett L. Tuttle and H. Tashiro, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

The European chafer, *Amphimallon majalis* (Raz.), is firmly established in several nursery areas of western New York. The promulgation of the Federal domestic quarantine in 1955 made it necessary to develop methods for eliminating all stages of the chafer in soil and about plant roots to satisfy the quarantine requirements.

One of the methods employed to free soil and plant material from infestation of all stages of the Japanese beetle is the use of heat. The immersion of subterranean portions of plants in water at 112° F. for 70 minutes is a certified quarantine treatment. Bulk soil must be heated to 130° for 30 minutes. Greenhouse operators commonly sterilize potting soil to control fungi, weed seeds, and other pests by subjecting it to a steam treatment at approximately 180° for several hours. Obviously, such treatment more than meets the minimum requirements for certification.

In 1956 tests were conducted at Geneva, New York, to determine the temperature-exposure relationships required for eliminating all stages of the European chafer. The methods employed by Fleming & Baker (1932) for determining this relationship for the Japanese beetle were used. All stages were immersed in water held at constant temperatures for various periods of time. Emphasis was placed on third-instar larvae, because they are present 9 months of the year and are thus more readily available for testing, as well as being a greater threat to dissemination than other stages.

Groups of 50 eggs or first-instar larvae were placed in 16×51 mm. glass tubes. The larvae were distributed between layers of cheesecloth to prevent cannibalism. Each end of the tubes was covered with a single layer of cheesecloth to confine the contents and allow free passage of water. The tubes were immersed in water at 112° and 130° F. for various intervals. Each treatment was replicated four times. As controls two tubes containing each stage were immersed in water at 64° for 40 minutes. After treatment the eggs and larvae were transferred to soil in ½-pint jelly glasses. The larvae were placed on paper toweling on the surface of moist soil. Those that crawled off the paper and into the soil were considered normal. A confirmatory re-examination was made in 48 hours. The eggs were placed in depressions on the soil surface and examined every other day for 4 weeks to remove larvae as they hatched. After completion of hatching, the soil was sifted to recover any larvae that had migrated into the soil.

Fifty third-instar larvae were placed individually in 1-inch cubicles of perforated metal trays and submerged in water held at several temperatures for various intervals. Each treatment was replicated at least twice.

Groups of 25 pupae were likewise separated in the perforated

[1] Accepted for publication August 14, 1958.

[2] In cooperation with the Department of Entomology, New York (Geneva) Agricultural Experiment Station.

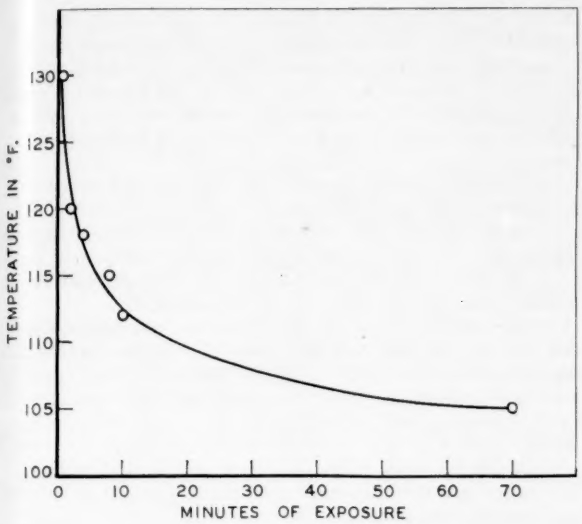


FIG. 1.—Time-temperature relationships required for elimination of third-instar European chafer larvae in hot water.

Table 1.—Mortality of the European chafer exposed to hot water.

WATER TEMPERATURE, (° F.)	EXPOSURE, (MINUTES)	PER CENT MORTALITY				
		Eggs	First Instar Larvae	Third Instar Larvae	Pupae	Adults
112	5	26	100	4	28	54
	10	32	100	100	100	100
	20	86	100	100	100	100
	40	100	100	100	100	100
Check (64)	40	19	11	—	4	4
130	1/6	—	96	—	54	10
	1/3	—	100	—	98	83
	1/2	100	100	100	100	93
	1	100	100	100	100	100
Check (64)	2	23	13	1	0	4

metal trays and immersed in water at 112° and 130° F. for various intervals. Adults were treated in the same manner as pupae in groups of 25 males and 25 females. They were no longer sexed when it became apparent that there was no difference in their susceptibility to hot-water treatment. Each treatment was replicated twice. Controls were held at 64° for at least 20 minutes. After treatment they were placed on the surface of moist soil in trays and examined for mortality. A confirmatory reexamination was made in 24 hours.

The time of exposure necessary to obtain complete kill of third-instar larvae ranged from 30 seconds at 130° to 70 minutes at 105° F. (fig. 1). First- and third-instar larvae, pupae, and adults were killed with a 10-minute exposure at 112°, but 40 minutes at this temperature was required to destroy all the eggs. All four stages of the chafer were killed with a 1-minute exposure at 130° F. (table 1).

These results demonstrated the adequacy of submerging underground parts of plants in water at 112° for 70 minutes, or maintaining a temperature of 130° for 30 minutes in the process of steam sterilization of bulk soil as a means of eliminating all stages of the European chafer.

The insecticidal action of heat is adequate to meet certification requirements for the European chafer quarantine.

REFERENCE CITED

Fleming, Walter E., and Francis E. Baker. 1932. Hot water as an insecticide for the Japanese beetle in soil and its effect on the roots of nursery plants. U. S. Dept. Agric. Tech. Bull. **274,** 41 pp.

Distribution of the Small Rice Weevil in the United States[1]

SYUNRO UTIDA, *Entomological Laboratory, Kyoto University, Kyoto, Japan*

Sasaki (1899) pointed out the occurrence of the small sized rice weevil in Japan as being different from the rice weevil, *Sitophilis oryza*, not only in size but also in biology. He named it *Sitophilus* (*Calandra*) *oryza* var. *minor*. Takahashi (1928) described it as *S. sasakii* pointing out the differences in biology as well as morphology. The damage caused by this species and by *S. oryza* is very serious in the main parts of Japan. Some of the workers in the author's laboratory have devoted attention for about 15 years to comparative studies of these two species from several different angles of approach; namely morphology, physiology, ecology, geographical distribution, habitat segregation, and cross breeding.

From the results of our experiment (Kono, unpublished), these two species are believed to be synonymous to the large and small strains of the rice weevil, which were reported by Birch (1944) and Richards (1944) from their occurrence in Australia and England, respectively. We now have living cultures of *Sitophilus sasakii* received from Australia, Argentina, Canada, China (Formosa), Nepal, and South Africa. It is believed that this species can easily be found in these districts as a grain pest. But, we have had no previous record of distribution in the United States, excepting the recent brief report of Floyd and Newson (1957).

During the past year, the author made a trip to the United States of America and to Canada, and had occasion to examine some preserved specimens of the rice weevil at several points in these countries. By examination of the morphological characters, especially of the pattern of the sculpture on the pronotum, some specimens of *S. sasakii* were found with those of *S. oryza* in the following collections: Chicago Natural History Museum, Chicago, Dr. Timberlake's collection in the Department of Biological Control, Citrus Experiment Station, Riverside, California, and Royal Ontario Museum, Toronto University. The following are the localities from which the specimens of *S. sasakii* were collected:

Honolulu, Hawaii (1924)
Lake Lucy, Florida (1925)
Wyandotte County, Kansas (1919)
Yolo, California (1896)
Winnipeg, Canada (1930)

A living culture of the weevil population thought to be *S. oryza* and which has been reared under laboratory conditions at the Department of Entomology, University of California at Riverside was brought to the author's laboratory and upon careful study of its biology and morphology was found to be the small rice weevil. We also know that the small rice weevil has frequently been found, as well as the rice weevil, in rice imported from the Southern United States to Japan. These results would seem to indicate fairly wide distribution of the small rice weevil, *S. sasakii*, in the United States, sharing the same habitat with the rice weevil, *S. oryza*.

The author wishes to express his appreciation to Prof. C. P. Clausen, Dr. H. Dybas and Dr. G. Wiggins, who allowed him

[1] Contribution from the Entomological Laboratory, Kyoto University, No. 305. Accepted for publication August 18, 1958.

to examine their insect collections when he visited them; also to Dr. D. L. Lindgren, who furnished the author a living culture of this weevil.

REFERENCES CITED

Birch, L. C. 1944. Two strains of *Calendra oryzae*. Australian Jour. Exptl. Biol. Med. Sci. **22**: 271–5.

Floyd, E. H., and L. D. Newson. 1957. Evidence of the occurrence of sibling species of rice weevils in the southern United States. (Abs.) Bull. Ent. Soc. Amer. **3**: 22.

Richards, O. W. 1944. The two strains of the rice weevil, *Calandra oryzae*. Trans. Roy. Ent. Soc. London **94**: 187–200.

Sasaki, C. 1899. Insects injurious to agricultural crops. (Japanese). Tokyo.

Takahashi, S. 1928. Insects injurious to field crops. (Japanese). Tokyo.

Persistence of Residues of Neotran on and in Mature Lemons and Oranges[1]

L. R. JEPPSON, L. M. ELLIOTT, and F. A. GUNTHER,
University of California Citrus Experiment Station, Riverside

Bis(*p*-chlorophenoxy)methane (Neotran) proved in laboratory and field studies by Jeppson in 1947 and 1951 to be very toxic to all stages (including eggs) of the citrus red mite, *Panonychus citri* (McG.), and its toxic effect persisted for several weeks after application to foliage and fruits. It was therefore of interest to establish magnitudes of residues on and in lemons and oranges as required by Public Law 518 (Miller Amendment). Residue data obtained by the total organic chloride combustion method described by Gunther & Blinn in 1955 on mature lemons and mature navel oranges are presented as a degradation study of Neotran. It is a characteristic of the total-chloride method that residue results represent maximum possible residues of this acaricide.

METHODS AND MATERIALS.—On November 26, 1956, mature lemon trees were sprayed with 20 ounces of 40% wettable powder per 100 gallons of finished spray mixture. Applications were made as conventional sprays, using a high-pressure reciprocating pump and manually operated spray guns. Final sprays were applied at the rate of approximately 1500 gallons per acre. On February 6, 1957, mature navel orange trees were sprayed with the same spray formulation at the rate of 2000 gallons per acre.

Mature fruit samples of both lemons and oranges were taken prior to the spray application and 1, 2, 4, 8, 16, and 37 days after treating the lemons, and 1, 2, 5, 9, 19, and 33 days after treating the oranges. Two fruits were picked from each quadrant of each of six trees in each plot, and the resulting 48 fruits were processed as a unit. Replicates were processed separately.

The unwashed fruits were peeled, and 1-pound subsamples of the minced peel were processed with petroleum ether as described by Gunther & Blinn (1955). Aliquots of the stripping solutions were washed free of inorganic chloride then assayed by the standardized combustion technique for total organic chloride.

Table 1.—Neotran residues expressed as parts per million on and in field-treated lemons and navel oranges.[a]

	Lemons				Oranges			
	Peel[b]		Pulp[c]		Peel[d]		Pulp[e]	
Elapsed Days	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
0[f]	0.2	—	—	—	0.0	—	—	—
1	14.1	15.2	—	—	14.8	11.4	—	—
2	15.6	15.2	0.0	0.0	12.5	12.2	0.0	0.4
4	14.8	14.4	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
8	12.2	10.6	—	—	12.9	—	—	—
9	—	—	—	—	11.0	10.6	—	—
16	9.9	10.0	0.0	0.0	—	—	—	—
19	—	—	—	—	9.1	10.8	0.0	0.0
33	—	—	—	—	6.8	8.4	0.2	0.4
37	9.5	8.7	0.0	0.0	—	—	—	—

[a] Infrared analysis of Neotran shows that soluble material is bis(*p*-chlorophenoxy) methane and accounts for 40±5% of the wettable powder formulation (Dow Chemical Company personal communication).

[b] Mature lemons have 30.0±8.5% peel from 632 measurements. Values corrected for recovery (97% from 12 samples fortified at 3.8 p.p.m.) and for average background from untreated control samples (0.2 p.p.m.).

[c] Edible portion of fruit. Values corrected for recovery (91% from three samples fortified at 3.0 p.p.m.) and for average background from untreated control samples (0.5 p.p.m.).

[d] Navel oranges have 22.1±7.3% peel from 567 measurements. Values corrected for recovery (89% from 11 samples fortified at 3.8 p.p.m.) and for average background from untreated control samples (0.2 p.p.m.).

[e] Edible portion of fruit. Values corrected for recovery (116% from three samples fortified at 3.0 p.p.m.) and for average background from untreated control samples (0.3 p.p.m.).

[f] Pretreatment samples.

RESULTS.—Residue values in peel and pulp of field-treated lemons and navel oranges are presented as parts per million of apparent Neotran in table 1.

DISCUSSION.—Previous residue studies for many insecticides and acaricides on and in citrus fruits have shown persistence half-life values in the range of 17 to more than 100 days (Gunther & Blinn 1955, 1956). Data herein presented, when plotted as a log curve of persistence, demonstrate that Neotran residues have a half-life of 30 days for lemons and 39 days for oranges.

REFERENCES CITED

Gunther, F. A., and R. C. Blinn. 1955. "Analysis of Insecticides and Acaricides." Interscience Publishers, New York-London.

Gunther, F. A., and R. C. Blinn. 1956. Persisting insecticide residues in plant materials. Ann. Rev. Ent. **1**: 167–80.

Jeppson, L. R. 1947. Di(4,chlorophenoxy)-methane for control of citrus red mite. Jour. Econ. Ent. **39**(6): 813.

Jeppson, L. R. 1951. Bis-(*p*-chlorophenoxy)-methane in relation to the control of citrus red mite and other mites injurious to citrus in California. Jour. Econ. Ent. **44**(3): 328–37.

[1] Paper No. 1045, University of California Citrus Experiment Station, Riverside, California. Accepted for publication July 21, 1958.

Toxicity of DDT in Oil and in Acetone to Adult DDT-Resistant House Flies[1]

ROY J. BARKER and LELAND N. EDMUNDS, JR., *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

When oil was used as a solvent for DDT instead of acetone, Busvine (1951) found less difference in tolerance to DDT between a susceptible and a resistant strain of house flies (*Musca domestica* L.). A susceptible strain tested by Barker and Rawhy (1957) had a higher regression coefficient of mortality on dosage for DDT in Risella oil than for DDT in acetone. Extrapolation of the log-dosage-probit lines suggests a point of intersection at high mortalities above which oil would be a more effective solvent than acetone. They did find that 0.8 μg. of DDT in oil killed flies of a strain that tolerated the same dosage in acetone. Hoskins and Gordon (1956) suggested that application of DDT in oil increases the accessibility of DDT and gives a more homogeneous response. This would be indicated by an increase in the slope of the log-dosage-probit line.

To discover a point of intersection of the log-dosage-probit lines, a moderately resistant strain (LDD) of 2- to 3-day-old

[1] Accepted for publication August 20, 1958.

Table 1.—Toxicity of DDT in oil and acetone to 2- to 3-day-old adult house flies of LDD strain, with limits for fiducial probability of 95%.

Solvent	Micrograms per Fly		Slope and Standard Error of of Log-Dosage-Probit Line
	LD-50	LD-95	
Males:			
Acetone	1.1 (0.8–1.4)	14.9 (9.8–22.6)	1.44 ± .15
Oil	2.5 (2.1–2.9)	7.8 (6.3– 9.8)	3.28 ± .34
Females:			
Acetone	4.5 (3.8–5.2)	47.6 (31.1–72.7)	1.60 ± .13
Oil	5.4 (4.6–6.3)	26.7 (18.0–39.6)	2.36 ± .30

house flies was treated topically with DDT in oil and in acetone. These flies had not been exposed to DDT for at least six generations. Treating procedures were essentially those of Barker and Rawhy (1957). The probit analyses were calculated at the Computing Center of Cornell University by its usual modification of the method of Finney (1952).

The results, given in table 1, show that the slopes for the LDD strain are much lower than those found previously for a susceptible strain of house flies. This finding supports the contention of Hoskins and Gordon (1956) that resistant strains have lower slopes after a few untreated generations. The slopes for Risella oil solutions again are steeper than those for acetone solutions. The slopes intercept at a dosage of 5.4 μg. with males and at 7.7 μg. with females. Above these dosages DDT is more effective in an oil solvent than in acetone; at lower dosages it is more effective in acetone.

The results emphasize that a single dosage is unsatisfactory in assaying relative potency or synergism; parallelism of the log-dosage-probit lines cannot be assumed. Compounds have been reported to be synergistic on resistant but not on susceptible insects (Perry & Hoskins 1951, Cohen & Tahori 1957). Since formulation changes the slopes of the log-dosage-probit lines, such materials as Risella oil could exhibit a pseudo-synergism at higher dosages by increasing the slope. This suggests that some formulations might obtain a more homogeneous response and retard the development of resistance to contact sprays.

References Cited

Barker, R. J., and A. Rawhy. 1957. Toxicity of DDT in acetone and in oil to adult house flies. Jour. Econ. Ent. **50:** 105.

Busvine, J. R. 1951. Mechanism of resistance to insecticides in house flies. Nature **168:** 193–5.

Cohen, S., and A. S. Tahori. 1957. Insecticide synergism, mode of action of di(*p*-chlorophenol)-(trifluoromethyl)-carbinol, as a synergist to DDT against DDT-resistant houseflies. Jour. Agric. Food Chem. **5:** 519–23.

Finney, D. J. 1952. Probit analysis. 318 pp. Cambridge.

Hoskins, W. M., and H. T. Gordon. 1956. Arthropod resistance to chemicals. Ann. Rev. Ent. **1:** 89–122.

Perry, A. S., and W. M. Hoskins. 1951. Synergistic action with DDT toward resistant house flies. Jour. Econ. Ent. **44:** 839–50.

Preplanting Dips Reduce Weevil Damage to Pine Seedlings[1]

R. C. Thatcher, *Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture*

Two species of weevils interfere seriously with regeneration of recently cut or burned pine lands in the Deep South. These insects, the pales weevil (*Hylobius pales* Hbst.) and the pitch-eating weevil (*Pachylobius picivorus* Germ.), kill pine seedlings by feeding on the bark and cambium (Sentell 1949, Thatcher 1957).

While southern sites become unattractive to these weevils in a year or 18 months after the pines have been cut or burned, immediate regeneration is highly advantageous to avoid encroachment by undesirable hardwoods.

Studies initiated in east Texas in 1955 indicated that dipping pine planting stock in 0.05 to 0.2% concentrations of wettable BHC reduced the mortality from weevils, but did not give complete protection (Ferguson & Thatcher 1956a, 1956b). The present study was undertaken in January 1957 to determine whether stronger concentrations would be more effective. Aldrin,[2] heptachlor,[3] and dieldrin[2] were also included, as they had shown promise in tests in the Southeastern States (Speers 1956).

Method.—The study plots were on the Stephen F. Austin Experimental Forest, near Nacogdoches, Texas. Twelve were in recently clearcut areas with varying weevil populations; they afforded a measure of the effect of preplanting dips in reducing weevil attack and seedling mortality. Three plots were in an open field, and were used to determine the effect of the treatment on seedling growth and survival in the absence of weevil populations.

In both woods and field plots, one-year-old loblolly pine (*Pinus taeda* L.) seedlings were planted in rows of 10. One row on each plot was randomly assigned one of the treatments. Two methods of application were tested—total dips, in which both tops and roots of the seedlings were immersed; and top dips, in which special care was taken to protect the roots from chemicals. In practice, total dipping is considerably easier than top dipping, as it requires less equipment and supervision. Nineteen treatments were established on each of the 12 woods plots and 23 treatments on each of the three field plots. All seedlings were examined frequently during 1957.

Results.—All chemical treatments reduced seedling mortality by amounts that statistical analysis showed to be significant at the 1% level (table 1). Most effective were the heavier BHC concentrations and total dips in aldrin emulsions and dieldrin suspensions. On the average, mortality was slightly higher for top-dipped seedlings than for those receiving total dips, but the differences were not significant.

Incidence of attack was not so consistently reduced. Dips in 0.5 to 2.0% BHC suspensions resulted in attack ratios that were very significantly less than those for untreated seedlings. Attack rates were lowered by other treatments also, though not significantly so.

Figure 1 shows cumulative seedling mortality trends for some of the more effective chemical treatments that demonstrated no phytotoxic effects. The trend for untreated seedlings is similar to that recorded in other studies (Thatcher 1957). The treatments materially reduced the steepness and height of the mortality curves. This indicates that, while incidence of feeding was not always significantly reduced, severity of damage by individual weevils was less extensive and therefore caused less mortality.

In the open field, little seedling mortality could be attributed

[1] Accepted for publication August 25, 1958.

[2] Aldrin WE-25 (an emulsifiable concentrate containing 2 pounds of aldrin per gallon) and Dieldrin 75-W (a wettable powder containing 75% of dieldrin) supplied by Thompson-Hayward Chemical Company, Houston.

[3] Heptachlor (transplant solution) supplied by Velsicol Chemical Corporation Chicago.

Table 1.—Summary of pine reproduction weevil attack and seedling mortality.

Per Cent Concentration, and Type of Dip	Clearcut Plots		Open field Plots	
	Total No. of Seedlings	Per Cent Attacked by Weevils	Total No. of Seedlings	Per Cent Seedlings Killed by Chemical
Check	120	49.2	30	0.0
BHC, suspension:				
0.2—top	120	41.7	30	.0
0.2—total	120	31.7	30	3.3
0.5—top	120	29.2	30	3.3
0.5—total	120	28.3	30	.0
1.0—top	120	22.5	30	.0
1.0—total	120	22.5	30	3.3
2.0—top	120	29.2	30	.0
2.0—total	120	10.8	30	40.0
Aldrin, emulsion:				
1.0—top	120	41.7	30	.0
1.0—total	120	35.8	30	6.7
2.0—top	120	40.0	30	.0
2.0—total	120	37.5	30	.0
Heptachlor, emulsion:				
1.0—top	120	38.5	30	.0
1.0—total	120	52.5	30	.0
2.0—top	120	47.5	30	.0
2.0—total	120	46.7	30	.0
Dieldrin, suspension:				
1.0—total	120	40.0	30	.0
2.0—total	120	50.8	30	.0
Dupont spreader-sticker				
top			30	.0
total				3.3
BHC, emulsion:				
1.0—total			30	.0
2.0—total			30	13.3

to the chemicals, except for 2% gamma concentrations of BHC when used as a total dip. Of all the seedlings dying from the various chemicals, only one had received a top dip. Height growth was very significantly reduced by total dips in both 1.0 and 2.0% BHC (wettable and emulsifiable).

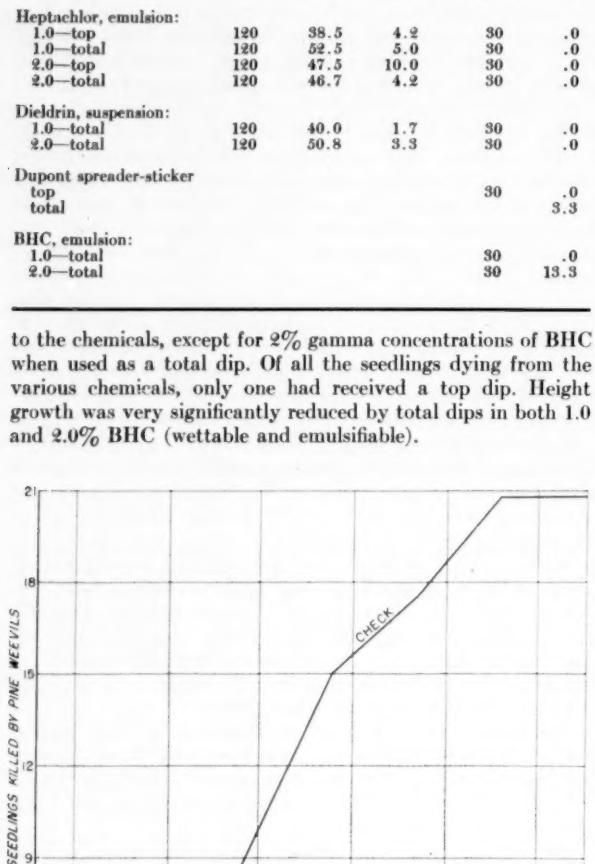


FIG. 1.—Effect of chemical dips in reducing loss of loblolly pine seedlings to pine reproduction weevils.

RECOMMENDATIONS.—For practical use, the best treatments may be to dip the tops of seedlings in a BHC suspension, or to dip both the tops and roots in an aldrin or heptachlor emulsion or in a dieldrin suspension. Concentrations of 1% appear adequate, and can be applied at low cost.

REFERENCES CITED

Ferguson, E. R., and R. C. Thatcher. 1956a. Preplanting dip for controlling pales weevil. Jour. Forest. **54:** 469–70.

Ferguson, E. R., and R. C. Thatcher. 1956b. Pales and his pals. Forest Farmer 15(11): 10–11, 18.

Sentell, N. W. 1949. Pales weevil damages plantations in Louisiana. Jour. Forest. **47:** 741.

Speers, C. F. 1956. Pales weevil control with insecticides: First year results. U. S. Forest Serv., Southeast. Forest Exptl. Sta. Res. Notes 96, 2 pp.

Thatcher, R. C. 1957. Pine reproduction weevils: Preliminary results from 1955–1957 studies. U. S. Forest Serv., South Forest Expt. Sta. South. Forest Pest Reporter **18,** 2 pp.

Disease-Producing Protozoa in Species of *Trogoderma*

F. O. MARZKE, *Dairy-Product Insects Laboratory*[1] and R. J. DICKE, *Department of Entomology, Wisconsin Agricultural Experiment Station,*[2] *Madison*

The apparent presence of disease was observed in cultures of *Trogoderma inclusum* Lec. at this laboratory in 1954. Samples of dead and unhealthy specimens were forwarded to the Insect Pathology Laboratory, Entomology Research Division, U. S. Department of Agriculture, in June and July 1955 and all showed protozoan infection. Three larvae of the first lot contained spores of a schizogregarine, two contained oocysts of a coccidian, and one contained both schizogregarine spores and coccidians. The second lot was infected with only the schizogregarine. At the suggestion of the Insect Pathology Laboratory, some exploratory transmission studies were conducted at Madison in August 1955, the results of which are presented in this paper.

Later the same schizogregarine organisms were found in larvae of *T. glabrum* (Hbst.) sent to the Insect Pathology Laboratory from the Stored-Grain Insects Laboratory, Manhattan, Kans. These larvae were collected from wheat in storage at Beloit, White City, Concordia, and Washington, Kans., during 1957. A high rate of infection with this schizogregarine has also occurred a number of times in *T. glabrum* cultures at the Manhattan laboratory.

Cultures of *T. glabrum* were sent from Manhattan to the Khapra Beetle Laboratory at Mesa, Ariz., for experimental studies. These cultures developed infection after arrival, and the infection spread to cultures of the khapra beetle, *T. granarium* Everts. Several samples of both cultures sent to the Insect Pathology Laboratory were found infected with apparently the same schizogregarine. The Khapra Beetle Laboratory reported that over 95% of the cultures of *T. glabrum* died and that approximately 55% of the *T. granarium* cultures were found diseased. The *T. granarium* seemed to show tolerance to the infection whereas the *T. glabrum* were virtually wiped out by the protozoan. Five other species, *T. inclusum*, *T. parabile* Beal, *T. teukton* n. sp., *T. simplex* Jayne, and *T. sternale* Jayne, were exposed to dead larvae and their food media which had

[1] This is a field station of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture. Acknowledgment is made to H. H. Walkden, of the Manhattan, Kans., laboratory, and G. H. Spitler, of the Mesa, Ariz., laboratory of this Section, for information relative to infections in *T. glabrum* and *T. granarium*.

[2] Approved for publication by the Director of the Wisconsin Agriculture Experiment Station. Accepted for publication September 6, 1958.

Table 1.—Percentage of live, moribund, and dead *Trogoderma inclusum* observed after various periods of exposure to culture media supplemented by ground sickly or dead larvae suspected of harboring organisms producing fatal infections

Length of Exposure (Weeks)	Noninfected Media				Noninfected Media Supplemented by Ground Sickly Larvae				Noninfected Media Supplemented by Ground Dead Larvae			
	Adults		Larvae		Adults		Larvae		Adults		Larvae	
	Alive	Moribund and Dead	Alive	Moribund and Dead	Alive	Moribund and Dead	Alive	Moribund and Dead	Alive	Moribund and Dead	Alive	Moribund and Dead
1	0	0	83	17	0	0	98	2	0	0	98	2
4	7	0	66	27	6	0	65	29	2	0	44	54
6	0	7	54	39	0	6	39	55	0	2	34	64
8	0	7	53	40	0	6	26	68	0	2	16	82
10	1	7	50	42	0	6	22	72	0	2	12	86
12	0	8	45	47	0	6	19	75	0	2	6	92

been found heavily diseased. The disease was transmitted to the first three species in varying degrees of severity, and the *T. simplex* and *T. sternale* were virtually wiped out.

These observations, and the results of the transmission tests, are presented here to record the infection of at least three species of the genus *Trogoderma* by this schizogregarine. The records suggest that this organism may have possibilities as a control measure for economically important species of *Trogoderma*.

TRANSMISSION TESTS.—Protozoa for these studies were obtained from dead and sickly larvae sifted from cultures of *T. inclusum* which appeared to be diseased; *i.e.*, more than 25% of the larvae were moribund or dead. Twenty-five larvae selected from apparently healthy cultures were then exposed continuously in petri dishes for 3 months to approximately 1½ ounces of one of the following media:

1. Freshly prepared Spratt's dog food, made by adding 5 grams of brewers yeast to each 95 grams of Spratt's Dog Food Special No. 24.

2. Same as 1 but supplemented by ground dead larvae from diseased cultures.

3. Same as 1 but supplemented by ground sickly larvae from diseased cultures.

At various weekly intervals all larvae were sifted from these media, and the number alive, moribund, and dead was recorded. The tests were concluded after 3 months. Each test was conducted in quadruplicate.

RESULTS.—High larval mortalities were observed in disease-free media, which would indicate that a number of larvae selected from cultures which appeared healthy may have harbored disease organisms. However, a significant increase in mortality was usually observed if the larvae were exposed to media supplemented by organisms from diseased cultures (table 1.)

After 6 weeks' exposure to freshly prepared media 39% of the larvae were moribund or dead, and in media supplemented by ground sickly or dead larvae 55 and 64%, respectively, were in a similar condition. After 12 weeks the percentage in freshly prepared media increased to only 47%, and in the media supplemented by ground sickly or dead larvae the percentage increased to 75 and 92%, respectively.

The protozoan parasites observed in sickly and dead larvae obtained from cultures of *T. inclusum* thus appear to be capable of producing a fatal infection which can be transmitted from one larva to another. The maximum increase in larval mortality occurred after exposure to media supplemented by ground dead larvae. The latter would undoubtedly contain the highest concentration of protozoan parasites. Further evaluation of the effectiveness of these pathogens in reducing populations in heavily infested areas would appear worthwhile.

Rearing *Sitona hispidula* Larvae for Various Research Uses[1]

R. C. NEWTON, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*

There is need for methods for rearing large numbers of larvae of the clover root curculio, *Sitona hispidula* (F.), for use in screening clovers and alfalfas for resistant plants, screening new insecticides, measuring root damage, and studying the relationship of this weevil and root rots in legumes. The writer has been using a method that produces larvae of fairly uniform size for studies throughout the winter and spring months. An average hatch of 78% was obtained for eggs incubated in cans in the laboratory. As many as 25% of the hatched larvae were recovered from the soil after 47 days, when they were about half grown. When these half-grown larvae were transferred to potted plants and the soil was examined 18 days later, 65% were recovered as full-grown larvae and pupae. This efficiency will undoubtedly be improved with further refinements in techniques. This method is described herein.

The procedure entails (1) providing food for the larvae and adults, (2) obtaining a supply of adults, (3) storing the adults, (4) setting up an oviposition chamber for collecting eggs, (5) incubating eggs, and (6) preparing the soil in potted plants for rearing larvae.

Buffalo alfalfa was used for food. It was planted in a greenhouse flat during the first week in September, and the seedlings were transplanted when 5 weeks old to 4-inch clay pots containing standard greenhouse soil. Plants were large enough to use for food by mid-November.

Adult weevils were swept from 14-inch alfalfa with a 15-inch diameter net from October 20 to 31. At this time from 2 to 74 were obtained per 200 sweeps, and a total of 800 were collected.

It is possible to collect some adult weevils in the late fall, winter, or early spring by the tedious method of examining the litter and soil close to the crowns of heavily infested alfalfa or red or Ladino clover, provided the fields are not snow-covered. Another method is to gather the plant litter and loose soil, scatter it lightly over wrapping paper in a warm room, and pick up the weevils as they crawl. A somewhat more effective method is to place the trash in a Berlese funnel in the laboratory and drive the weevils out with heat. Another possibility is to collect litter and about ½ inch of the topsoil from fields, work the material through ¼- and 16-mesh screens, wrap the residue on the 16-mesh screen in paper toweling until dry, and then examine it for adults.

[1] Accepted for publication September 6, 1958. Contribution No. 159 of the U. S. Regional Pasture Research Laboratory, University Park, Pa. This laboratory is operated by the Crops Research Division in cooperation with the Entomology Research Division and the Northeastern States.

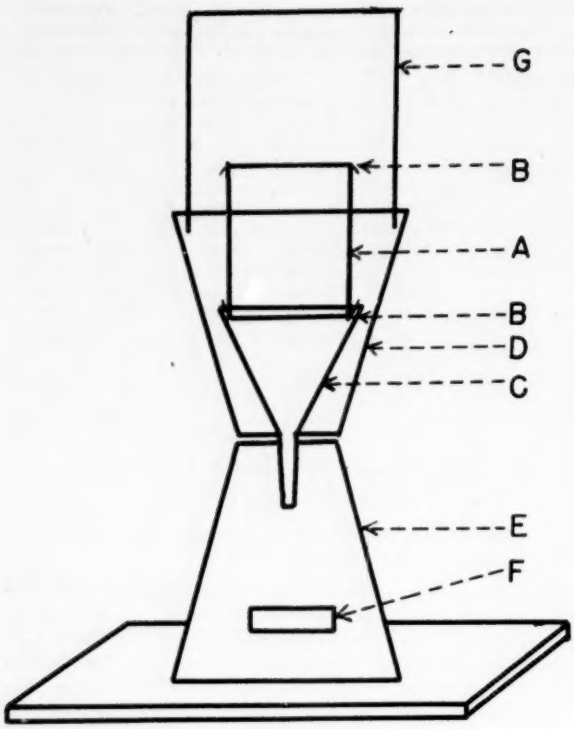


FIG. 1.—Assembled oviposition chamber: *A*, Lamp chimney containing *Sitona* and food; *B*, nylon cloth held in place by elastic bands; *C*, funnel supported by flowerpot, *D*, placed over inverted pot, *E*; *F*, can for collecting eggs; *G*, glazed crock used as cover.

FIG. 2.—Feeding injury to alfalfa roots by larvae of *Sitona hispidula*. Right, infested plant; left, healthy plant.

Adult weevils were kept in lots of 200 in cottage-cheese containers 4½ inches in diameter to which moist soil and a few fresh alfalfa stems were added. A $\frac{3}{16}$-inch hole punched in the cover and kept closed with adhesive tape facilitated putting the weevils in the box without permitting others to escape. The boxes were then stored in the refrigerator at 40° F. Adults thus stored did not oviposit, but did some feeding so that it was not necessary to add moisture and fresh plants more than twice a month. It was found that eggs could be obtained at any time up to 5 months by removing a portion of the October-stored adults from refrigeration and holding them at room temperature.

To obtain eggs about 25 adults of both sexes were confined in an oviposition chamber (fig. 1) with fresh food. The chamber consisted of a 5-inch-diameter straight-sided Coleman lamp chimney, both ends of which were covered with 30-mesh nylon marquisette held in place with elastic bands. One end of the lamp chimney was placed down into a funnel 5½ inches in diameter which was supported inside an 8-inch flowerpot. This pot was placed over an identical inverted pot covering an ointment can 3 inches in diameter and 1 inch deep so that the eggs fell through the nylon cloth into the funnel and can. The top pot was kept covered with an inverted 7½-inch diameter glazed crock to prevent the cut alfalfa from drying. The clay pots could be soaked in water to provide increased humidity if necessary.

Eggs were collected daily in tin cans containing moist plaster of Paris. When these cans were kept covered and the eggs incubated at room temperature, the per cent of total hatch based on 261 eggs was as follows: 8th day 7.3, 9th day 31.8, 10th day 42.9, 11th day 13.8, 12th day 3.4, 13th day 0.4, and 17th day 0.4.

When the eggs were 7 days old, they were carefully swept with a camel's-hair brush onto the moist soil around a potted alfalfa plant. About 12 to 14 holes had previously been made in the soil around the plant by pushing a ⅛-inch diameter brush handle about 2½ inches into the root area. Transferring the eggs just before hatching permitted them to hatch undisturbed, and the holes made it possible for the small larvae to penetrate the soil and find roots with a minimum of effort. The plant top was then cut back to about 4 inches, and the pot was completely wrapped in aluminum foil by cutting an X in a sheet and slipping it over the plant and pressing it along the sides of the pot. A second sheet was used to seal the entire bottom and sides of the pot. Plants with larvae were watered only when necessary, and then sparingly through the opening in the top so that the soil was only slightly moist at all times. Pots were kept in the greenhouse at about 60° to 65° F. Larvae became about half grown in 45 days, and started pupating in 65 days. They were recovered by slowly sifting the soil through 14- and 30-mesh screens onto wrapping paper, and examining the screens and soil.

An examination of the roots of potted plants used in rearing *hispidula* grubs dramatically showed the type and extent of damage they are capable of inflicting. A total of 56 half-grown grubs were transferred to an uninfested pot of Buffalo alfalfa for 18 days. When the soil and roots were later examined, 32 larvae and pupae were recovered. At least half of the plant rootlets and secondary roots had been consumed, and the main roots showed numerous feeding scars and gouged areas. This partly eaten root system compared with that of a healthy plant is shown in figure 2.

Although the rearing efficiency of this method leaves room for improvement, large numbers of *hispidula* eggs can be obtained easily by increasing the numbers of adults confined in the oviposition chamber, running several chambers simultaneously, and using more potted plants.

Control of the Seed-Corn Maggot on Radishes

F. B. FOLEY and M. W. STONE, *Entomology Research Division Agr. Res. Serv., U.S.D.A.*

In December 1956 attention was called to severe damage in a commercial planting of radishes near San Juan Capistrano, Calif. An examination showed that 21% of the radishes were injured by the feeding of the seed-corn maggot, *Hylemya cilicrura* (Rond.).

In southern California radishes mature in 45 to 50 days and crops are grown continuously in the same field. Infested radishes from each preceding crop remain to build up maggot populations. It appeared desirable to test insecticides that had been used successfully in soil applications against the seed-corn maggot in turnips (Stone & Foley 1957).

The experiments were conducted in a commercial field of Southern Market Globe radishes in plots arranged in randomized blocks with 5 or 6 replicates of each treatment. Insecticides were applied to plots 6×7 feet with hand sprayers (Expt. 1), and plots 15 to 30 feet wide by 90 feet long with a power sprayer (Expts. 2 and 3). They were applied as emulsions on the soil surface at rates of 34 to 100 gallons per acre and disked in 4 to 5 inches deep on the same day. The applications were made 1 to 3 days before planting. Their effectiveness was determined by pulling mature radishes at random from each plot and checking for injury. Radishes with one or more feeding scars or holes were classified as being injured or unmarketable. The experiments were conducted from December 3, 1956, to April 15, 1957, when maggots and adults were most numerous. In Experiment 3 a second crop of radishes was planted, and these were examined for injury in February 1958. The results are presented in table 1.

Table 1.—Control of the seed-corn maggot on radishes with various insecticides, San Juan Capistrano, Calif.

INSECTICIDE	POUNDS PER ACRE	PER CENT OF RADISHES DAMAGED
Experiment 1 (5 replicates, 700 radishes)		
Heptachlor	2	4.3
	4	2.9
	8	2.3
Dieldrin	2	4.6
	4	4.1
	8	1.3
DDT	20	11.6
Untreated	—	28.4
L.S.D. at 5% level	—	2.1
Experiment 2 (6 replicates, 600 radishes)		
Heptachlor	3.0	2.7
	6.0	2.7
Dieldrin	2.8	4.0
	5.6	1.8
Endrin	3.0	2.0
	6.0	4.2
Untreated	—	9.0
L.S.D. at 5% level	—	3.3
Experiment 3 (6 replicates, 600 radishes)		
Heptachlor	2.7	1.3, 3.0[a]
	5.3	0.5, 3.0
Dieldrin	2.8	2.0, 3.0
	5.6	1.2, 4.3
Endrin	2.8	3.3, 2.7
	5.6	1.2, 2.0
Toxaphene	23.0	6.8, 7.3
Untreated	—	20.5, 8.3
L.S.D. at 5% level	—	2.8, 3.1

[a] First and second crops, respectively.

In Experiment 1 heptachlor, dieldrin, and DDT were applied on December 3 and the radishes were examined on January 21 and February 4. The 2- and 4-pound dosages of heptachlor and dieldrin gave commercial control (not more than 5% of the crop damaged), and the 8-pound dosage of dieldrin was highly effective, but DDT at 20 pounds gave partial but unsatisfactory control.

In Experiment 2, dieldrin, heptachlor, and endrin were applied on January 31. Examination of radishes on March 20 showed no significant differences in control between treatments, but all materials were responsible for significant reductions in injury below the check.

In Experiment 3 heptachlor, dieldrin, endrin, and toxaphene were applied on February 20. Radishes from two successive crops were examined on March 25 and May 10. In both crops all materials gave significant reductions in injury below the check, and all except toxaphene gave commercial control.

In these experiments heptachlor, dieldrin, and endrin, in that order, gave commercial control of the seed-corn maggot on radishes but toxaphene and DDT were less effective.

REFERENCE CITED

Stone, M. W., and F. B. Foley. 1957. Control of soil insects on turnips. Jour. Econ. Ent. **50**(2): 143–5.

[1] Accepted for publication September 6, 1958.

A Technique for Rapid Rearing of Clothes Moth Eggs[1]

ROY J. PENCE, *Department of Entomology, University of California, Los Angeles*

Investigation of the dietetics and use of antimetabolites in the nutritional requirements of fabric pests indicated a need for limited but rapid egg production of these insects.

As most of the preliminary work in this endeavor is being carried out with the webbing clothes moth, *Tineola bisselliella* (Hum.), it became necessary to devise a technique for rapid production of adequate numbers of clean eggs. Various methods of large-scale production of clothes moth eggs have been developed in past investigations (Heal 1942). These procedures are used by commercial laboratories where the need for test insects is extensive (Mallis *et al.* 1958). Such production, however, necessitates a staff of trained personnel to maintain continuous vigilance and service.

The need for rapid egg production on a more modified scale resulted in the development of the technique described herein.

Cultures of webbing clothes moth larvae are reared in much the same manner as recommended under Chemical Specialties Manufacturers Association standards. The cultures are propagated on a medium of loose rolls or strips of clean woolen felt. The fabric is nutritionally enriched by the addition of powdered brewer's yeast (Colman 1922, Frankel & Blewett 1946). The medium is kept in one-quart, wide-mouthed Mason jars with open-centered screw-type lids. A circular piece of filter paper or paper toweling is secured in place by the screw-type lid to allow

[1] Accepted for publication September 6, 1958.

FIG. 1.—Webbing clothes moth culture jar inverted over oviposition jar containing sheets of black photographic paper and shallow medium of 50-mesh yeast and dog food.

ventilation and prevent escape. Cultures are matured in a cabinet thermostatically controlled to maintain a temperature of 80° F. and a relative humidity of 65%. The maturing larvae, through the process of webbing, will fasten the fabric securely to the sides and bottom of the jars. Thus the jars may be inverted to permit passage of the adults to another jar without loss of the rearing medium.

Jars used for oviposition are identical to the rearing jars; however, the medium used for this purpose consists of one-half dried dog-food meal and one-half brewer's yeast, which is pulverized until it passes through a 50-mesh screen. Enough of this finely-divided powder is poured into the bottom of a clean oviposition jar so that, after settling, it reaches a depth of approximately ¼ inch.

Black photographic paper is cut into sheets 2½ inches wide and 4 inches long. Five to six of these sheets are placed on end and pushed down into the medium until contact with the bottom of the jar is reached. The sheets should be spaced far enough apart so as to allow adult moths to pass freely between them.

Clothes moths are introduced into the prepared oviposition jars by inverting a jar containing the matured culture over the opening. As the jar openings will match, the inverted culture jar may be allowed to rest in this position for an indefinite period. A few raps, however, will disturb the adults and cause them to fly downward into the oviposition jar. As clothes moths are negatively phototropic, the aid of a strong light directed over the culture will drive the remaining adults downward where they will quickly seek the protection of the black photographic paper beneath (fig. 1). Pairing and oviposition then take place.

The advantage of this technique lies in the ease of handling of each sheet of black photographic paper. Within 2 to 3 days the gravid female moths will deposit their eggs in even lines at the juncture of the pulverized medium and paper. As the white eggs are also laid at random over the surface of the black paper

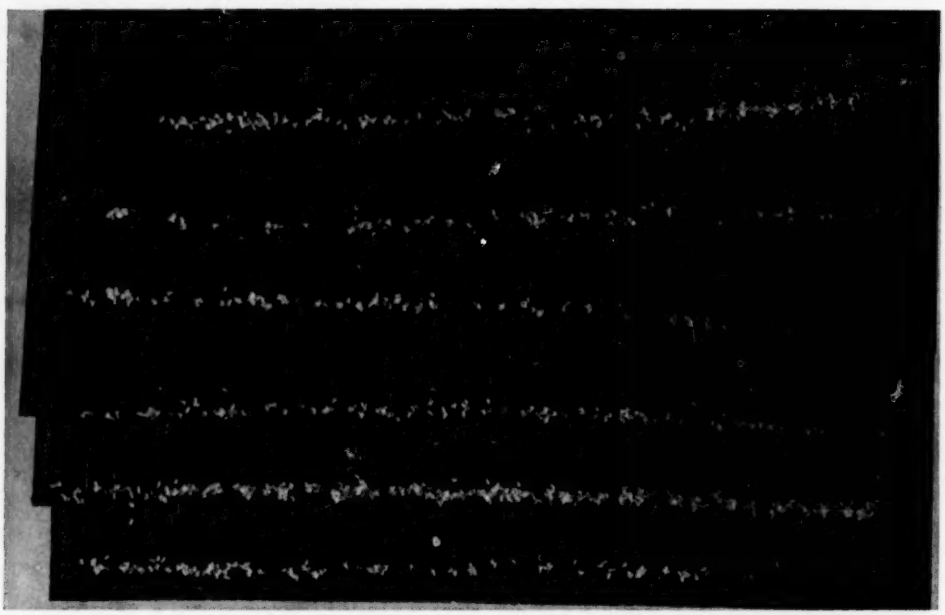


FIG. 2.—Webbing clothes moth eggs deposited in straight lines where black photographic paper and yeasted ground dog food intersect in oviposition jar.

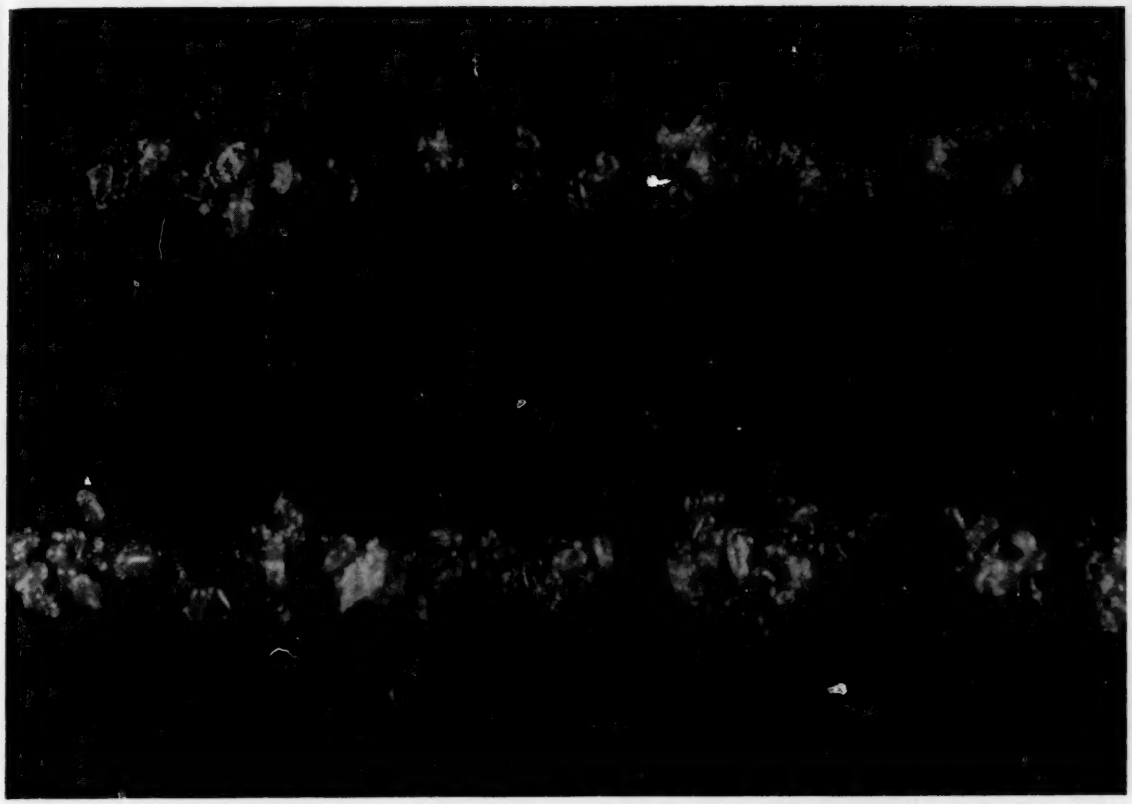


FIG. 3.—Close-up of webbing clothes moth eggs deposited on black photographic paper.

sheets, they may be easily detected and brushed free without damage, and any infestation of mites becomes greatly lessened in the process (figs. 2 and 3).

The attraction of the medium also causes eggs to be laid over its surface. Once the paper sheets have been brushed free of eggs the medium is once again passed through the 50-mesh screen. As the eggs are too large to pass through, they are trapped on the surface in a pure state and added to those already claimed from the papers. The medium and paper are then ready for re-use.

REFERENCES CITED

Colman, W. 1922. Effect of yeast on clothes moth larvae. Jour. Econ. Ent. **25**(6): 1242.

Fraenkel, G., and M. Blewett. 1946. The dietetics of the clothes moth. Jour. Exptl. Biol. **22**(3 & 4): 156–61.

Heal, Ralph E. 1942. Evaluating protection of fabrics from clothes moth and carpet beetle attack. Jour. Econ. Ent. **35**(2): 249.

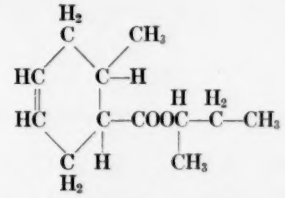
Mallis, Arnold, A. C. Miller, and R. C. Hill. 1958. Feeding of four species of fabric pests on natural and synthetic textiles. Jour. Econ. Ent. **51**(1): 248.

Effect of *cis-trans* Isomerism on the Potency of an Insect Attractant[1]

L. F. STEINER, W. C. MITCHELL, NATHAN GREEN and MORTON BEROZA, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

In the 1956–57 Florida campaign to eradicate the Mediterranean fruit fly, *Ceratitis capitata* Wied., chemical attractants and traps were depended upon to detect and delineate infested areas and to determine when eradication was attained.

Angelica seed oil, derived from *Archangelica officinalis* Hoffn., which had been found to be an outstanding Mediterranean fruit fly attractant (Steiner *et al.* 1957), was used extensively in the campaign until the world supply neared exhaustion. Meanwhile, certain esters were shown (Gertler *et al.* 1958) to be effective lures for this insect in Hawaii and they subsequently played an important role in the Florida campaign. These synthetic lures had the advantage of being more readily available in a state of controlled purity than angelica seed oil. When used in plastic traps at six times the rate of angelica seed oil, they often gave equal catches (Steiner 1957). The best of the esters and the one most used in Florida was *sec*-butyl 6-methyl-3-cyclohexene-1-carboxylate (I).



I

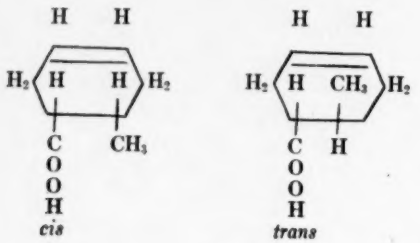
Olfactometer and field tests conducted in Hawaii to assay commercially produced lots of this ester indicated that substantial differences in attractiveness existed between laboratory and the commercial batches. In 27 replicated field experiments, each of 7 days' duration, the laboratory batches at approximately 3 ml. per trap gave catches averaging 84% of those made

[1] Accepted for publication September 12, 1958.

[2] Doris H. Miyashita assisted in the olfactometer studies and R. N. Kinoshita and T. Urago in the field studies.

by angelica seed oil at 0.5 ml. per trap. The effectiveness of 33 lots of the commercial product ranged from 16 to 100% of that of the laboratory lots in nearly 100 experiments yielding more than 75,000 Mediterranean fruit flies. This variation proved puzzling because of the uniformity of the several batches as indicated by the usual physical and chemical tests. An intensive study of fractions and isomers was then undertaken to determine whether *cis-trans* isomerism was related to attractiveness.[3]

6-Methyl-3-cyclohexene-1-carboxylic acid (as well as its esters) can exist in two isomeric forms, which may be depicted as follows:



II

A *cis*-rich acid, prepared by a fractional-crystallization process, had an isomeric assay of 69% *cis* and 31% *trans*. In a 3-week field test its *sec*-butyl ester proved significantly inferior (782 flies caught) to the corresponding all-*trans* ester (1117 caught). Later a method was devised for analyzing and separating mixtures of the two isomeric acids so that the synthesis of the corresponding *sec*-butyl esters was possible (Green & Beroza, unpublished manuscript). The pure *cis* ester was compared in the Gow olfactometer (Gow 1954) with previously tested samples. The numbers of Mediterranean fruit flies attracted in 1-hour exposure are shown below:

cis 100%	150
cis 69%, trans 31%	300
trans 100%	400

The laboratory batches of the *sec*-butyl ester proved to be essentially pure *trans*, having been prepared under very mild conditions from the pure *trans* acid. Commercial samples, which averaged 70% *trans*, were somewhat less attractive. Special commercial production, designed to provide the maximum content of *trans* isomer, resulted in an attractant that caught 35,000 Mediterranean fruit flies compared with 37,000 taken by standard angelica seed oil in paired tests extending over a 6-month period and involving eight or more replicates. The same improved product, in 8 weeks of paired tests, caught 25,500 flies compared with 17,600 taken in traps baited with the original commercial preparation.

It is believed that the tests herein reported represent the first comparison of the *cis* and *trans* isomers of an effective insect attractant.

REFERENCES CITED

Gertler, S. L., L. F. Steiner, W. C. Mitchell, and W. F. Barthel. 1958. Esters of 6-methyl-3-cyclohexene-1-carboxylic acid as attractants for the Mediterranean fruit fly. Jour. Agric. Food Chem. **6**(8): 592–4.

Gow, P. L. 1954. Proteinaceous bait for the oriental fruit fly. Jour. Econ. Ent. **47**(1): 155–60.

Steiner, Loren F., D. H. Miyashita, and L. D. Christenson. 1957. Angelica oils as Mediterranean fruit fly lures. Jour. Econ. Ent. **50**(4): 505.

Steiner, Loren F. 1957. Low-cost plastic fruit fly trap. Jour. Econ. Ent. **50**(4): 508–9.

[3] H. L. Haller, Assistant Administrator, Agricultural Research Service, suggested the studies of the *cis* and *trans* isomers.

NOTICE TO MEMBERS

A new periodical, *Miscellaneous Publications of the Entomological Society of America* will be initiated by the Society in 1959. It will be devoted to entomological articles of more than 20 printed pages and of a type and length less than that suitable for the Thomas Say Foundation series.

If you have questions or a suitable manuscript please write the Executive Secretary, Entomological Society of America, 1530 P Street, N. W., Washington 5, D. C.

NOTICE TO NON-MEMBER SUBSCRIBERS

Beginning in 1959, subscriptions to the JOURNAL OF ECONOMIC ENTOMOLOGY and to the *Annals of the Entomological Society of America* will be $15.00 each per year, domestic; $15.60 each per year, foreign. A single order for both publications, if sent to the same address, may be obtained at a COMBINATION PRICE of $27.00 per year, domestic; $28.20 foreign.

Index, Volume 51, 1958

Acalymma vittata, 81
Acarina, 259
Acaridiae, 767
Aceria
sheldoni, 657
tulipae, 68, 303
Acetals, as synergists in house fly sprays, 39
Acetates, myristyl and cetyl bromo- and chloro-, speed of action on resistant house flies, 880
Acethion and related compounds, toxicity, hydrolysis, and anticholinesterase activity of, 716
Achaea ablunaris, 637
Achaetoneura archippivora, 562
Achatina fulica, 390
Acheta assimilis, 866
Acids, bromo-, chloro-, and iodoacetic, speed of action on resistant house flies, 880
Acrospidia myrmicoides, 92
Acrostalagmus, probably *aphidium*, 184
Acrosternum
herbida, 636
vicina, 636
Acrylonitrile, dosages needed to kill rice weevil, 157
Adalia, 614
bipunctata, 81
Adelphocoris
lineolatus, 493, 729
rapidus, 493
Aedes
aegypti, 126, 187, 239, 345, 409, 499, 744, 780
atlanticus, 846
infirmatus, 846
sollicitans, 846
spp., 845
taeniorhynchus, 640, 846
triseriatus, 744
zoosophus, 744
Aedes, floodwater species of, 56
Aeolothrips duvali, 450
Aestivation of the alfalfa weevil, 506
Agathis stigmaterus, 164
Agonoderus comma, 81
Agriotes mancus, 690
Aircraft, jet, insect survival in, 239
Alabama argillacea, 237, 259, 633, 758, 783
Aldrin
as a fumigant against house flies, 638
as a seed treatment against wireworms, 294
dips to reduce weevil damage to pine seedlings, 916
distribution of in soils, 381
Aldrin, conversion to dieldrin,
in soil, 1
on plants, 8
Aldrin, for control of
alfalfa snout beetle, 683
carrot rust fly, 166
clover leaf weevil, 195
European chafer, 766
Nysius raphanus, 251
onion maggot, 884
paddy borer, 571
plum curculio, 131
serpentine leaf miner, 358
sugarcane beetle, 631
weevils on crimson clover, 459
western subterranean termite, 209
wireworms, 464, 691
Aldrin
granules, performance of broadcaster with, 280
oral chemotherapeutic activity of in mice, 127
percentage recovered from treated soil, 201
persistence and transformation of in soils, 214
residues of in carrots, 337
residues on alfalfa, 222
Aldrin, toxicity of to
army cutworm, 726
brown-banded cockroach, 619
Ceratitis capitata, 235
pink bollworm adults, 578
young cabbage and muskmelon plants, 534
Alfalfa
insect-free, seed production and growth characteristics of, 729
reaction of to spotted alfalfa aphid, 601
resistant to meadow spittlebug, 219
Alkoxybenzyl esters of chrysanthemumic acid, toxicity of in house fly sprays, 810
Allethrin
cis isomers of, mixed with piperonyl butoxide, 675
four *cis* isomers of, 181
toxicity of in house fly sprays, 810
with acetal synergists in house fly sprays, 39
Allethrin
cis and *trans* fractions of,
intensity of synergism of their mixtures with piperonyl butoxide, 76
relative toxicity to house flies, 76
Allotropa citri, 90
Alphitobius diaperinus, 112
Altica ambiens, 296
Amblyomma
americanum, 425
maculatum, 499
Amblyseius sp., 366
Am. Cyanamid 4124 (see also dicapthon)
for control of bed bug, 241
toxicity of to honey bees, 103
Am. Cyanamid 12008, for control of
Aceria tulipae, 70
greenbug, 723
green peach aphid, 139
spotted alfalfa aphid, 122
thrips on cotton, 160
two-spotted spider mite, 736
Am. Cyanamid 12008
metabolism of by some soil organisms, 59
toxicity of to honey bees, 103
translocation and persistence of in cacao foliage, 773
Am. Cyanamid 12009, for control of
Aceria tulipae, 70
thrips on cotton, 160
Am. Cyanamid 12009
toxicity of to honey bees, 103
translocation and persistence of in cacao foliage, 773
Am. Cyanamid 12880,
as a chemotherapeutic agent, 126
bioassay of blood from cattle treated with, 861
Am. Cyanamid 12880, for control of
balsam gall midge, 400
cattle grubs, 445, 583, 793, 876
cattle lice, 876
lone star tick, 425
screw-worm, 425
stable fly, 425
Am. Cyanamid 12880, radioactive, in mosquitoes, 640
Am. Cyanamid 18706, for control of two-spotted spider mite, 736
Ammonium carbonate to attract walnut husk fly, 687
Amorbia essigana, 296
Amphimallon majalis, 486, 765, 869, 912
Anagyrus
antoninae, 363
diversicornis, 365
graminicolens, 365
kivuensis, 92
pseudococci, 90
Analyses
microbial, from insecticide-treated soils, 200
physical, chemical, and microbial of Chehalis and Olympic silt loam soils, 204
probit, on a digital computer, 738
Anaphothrips zeae, 450
Anarhopus sydneyensis, 92
Anasa tristis, 169
Anastatus blattidarum, 608
Androlaelaps sp., 366
Anoetus sp., 461
Anopheles
barberi, 744
quadrimaculatus, 240, 345, 640
Anthonomus
grandis, 41, 93, 100, 114, 236, 288, 502, 508, 669, 719, 745, 783, 872
sp., 636
Anthrenus flavipes, 248
Anticarsia gemmatalis, 636
Anticholinesterase agents, recovery of from cacao beans treated with,
BHC, 778
Chipman R-6199 (free base), 777
Chipman R-6200, 778
demeton, 777
dimefox, 778
Isolan, 777
Thimet (Oxygen analog sulfone), 777
Antonina
graminis, 363
indica, 363
phragmitis, 365
purpurea, 365
Anuraphis roseus, 354
Anystis sp., 366
Aonidiella aurantii, 193, 232, 254, 474, 764 911
Apanteles
angaleti, 164
chelonis, 390
Aphelinus
nigritus, 553
semiflavus, 374
Aphicides, selective, favoring natural enemies of the spotted alfalfa aphid, 374
Aphidius testaceipes, 553
Aphids

control of on cotton with systemics, 699
effect of low temperatures on survival of, 144
populations on field beans in Washington, 178
provide substitute sugar source for honey bees, 287
Aphis
abbreviata, 144, 414
crataequifoliae, 179
fabae, 735
gossypii, 41, 45, 236, 636, 872
helichrysi, 226
knowltoni, 179
phaceliae, 179
pomi, 357, 422
spiraecola, 179
Aphodius distinctus, 81
Aphytis
chrysomphali, 476
lingnanensis, 476
Apis mellifera, 46, 64, 103, 146, 465, 494, 523, 784
Aramite, for control of
chicken body louse, 230
citrus bud mite, 659
cyclamen mite, 432
mites attacking eye gnat cultures, 462
two-spotted spider mite, 353, 356, 711, 714
Aramite, relative toxicity of to five species of tetranychid mites, 521
Aramite, resistance to by
Tetranychus atlanticus, 286
Tetranychus pacificus, 286
Archips
argyrospila, 598
rosana, 89
Archytas marmoratus, 562
Argyrotaenia
citrana, 89, 596
velutinana, 81, 357, 378, 454
Arhyssus parviconis, 636
Aroclor, for control of house flies, 20
Arthrochlamps plicata, 81
Aspidiotiphagus citrinus, 115
Artemia salina, use of for bioassay, 780
Asca
bicornis, 770
sphidioides, 770
Aster-yellows virus infection and transmission, 512
Astymachus sp., 366
AT-4, for control of horn flies and stable flies, 73
Athaumastus haematicus, 636
Attaclay, as a carrier for insecticides against wireworms, 463
Attagenus piceus, 248, 249, 887
Attractant, insect, effect of *cis-trans* isomerism on the potency of, 921
Autoserica castanea, 547
Attractants
for walnut husk fly, 689
glycine-sodium hydroxide solution for walnut husk fly, 532

Bacillus thuringiensis, 390
for control of codling moth, 422
susceptibility of some insects to, 296
Baits
attractants for walnut husk fly, 689
against house flies, 19
Dipterex against house flies, 19, 798
DDVP against house flies, 19
DDT against confused flour beetle, 301
for control of *Fannia canicularis*, 611
for control of *Leptocoriza acuta*, 405
lures for Mediterranean fruit fly in Florida, 679
malathion against house flies, 19
Pival against confused flour beetle, 301
rodenticidal, resistance of to *Tribolium confusum*, 299
synergized pyrethrins and allethrin to control house flies, 798
Balaustium sp., 366
Barichneumon, 652
Bathyplectes curculionis, 298
Bayer 13,242, systemic activity against the bed bug, 499
Bayer 17,364, systemic activity against the bed bug, 499
Bayer 18,613, systemic activity against the bed bug, 499
Bayer 19,596, systemic activity against the bed bug, 499
Bayer 19,639
for control of boll weevil, 100
citrus whitefly, 552
cotton aphid, 100
iris borer, 550
onion maggot, 885
spotted alfalfa aphid, 122, 457
thrips on cotton, 160
two-spotted spider mite, 736
Bayer 19,639
effect of seed treatments on cotton, 495, 697
resistance of citrus red mite to, 233
tests with, as cottonseed treatments, 872
translocation and persistence of in cacao foliage, 773
translocation of in pineapple plants, 905
Bayer 19,994, systemic activity against the bed bug, 499
Bayer 21/199, fate of in the white rat, 204
Bayer 21/199, for control of
cattle grubs, 359, 545, 583, 793, 876
cattle lice, 876
horn flies, 559, 746, 795
lone star tick, 425
onion maggot, 886
screw-worm, 408, 425
stable fly, 425
Bayer 21/199
radioactive, in mosquitoes, 640
resistance of house fly to, 497
Bayer 23,129, for control of
lone star tick, 425
screw-worm, 425
spotted alfalfa aphid, 123, 457
stable fly, 425
two-spotted spider mite, 736
Bayer 23,129, translocation and persistence of in cacao foliage, 773
Bayer 23,453, for control of
lone star tick, 425
screw-worm, 425
stable fly, 425
Bayer 25,141, for control of boll weevil, 785
Beauveria bassiana, 391
Behavior, wireworm, in response to chemical seed treatments, 291
Bergoldia clistorhabdion, 454
BHC dips to reduce weevil damage to pine seedlings, 916
BHC, effect of on
parasites of oriental fruit moth, 85
soft scales on walnut, 429
BHC, for control of
alfalfa snout beetle, 683
apple grain aphid, 355
boll weevil, 784
cotton leafworm, 784
Epilachna vigintioctopunctata, 749
European chafer, 766
greenbug, 723
peach tree borer, 558
root collar borer in yellow-poplar, 252
rosy apple aphid, 355
serpentine leaf miner, 358
walnut scale, 528
BHC, polyfluoro analogs of, 413
BHC, residues in treated soil, 187
BHC, toxicity of
in milk to house flies, 560
to pink bollworm adults, 578
to *Therioaphis maculata* and *Hippodamia*, 377
Bioassay
for testing effectiveness of malathion and lindane against wheat insects, 843
of carrots for aldrin residue, 337
to determine concentration of Bayer 19,639 in pineapple plants, 905
to determine insecticide residues in soils, 186
to determine persistence of aldrin and heptachlor residues on alfalfa, 222
to determine residue of dieldrin on alfalfa, 685
to study decline of soil insecticides, 200
use of brine shrimp as a test organism, 780
with stable fly, of blood from cattle treated with Am. Cyanamid 12880, 861
Biological Control
agents introduced into Indonesia, 390
characteristics of Ichneumonidae in relation to, 650
Rhodes-grass scale in Texas, 363
Biological effectiveness of
insect screens, 864
Biology of
Aphis helichrysi, 226
brine shrimp, 781
brown-banded cockroach, 608
carrot weevil in Illinois, 37
cherry fruit flies on Vancouver Island, 592
citrus cutworm, 653
Euzophora ostricolorella, 251
Fiorinia externa, 405
McDaniel mite in Utah, 588
purple-backed cabbageworm, 281
seed-corn maggot, 704
vetch bruchid, 401
Bio 1240, resistance of citrus red mite to, 232
Black Leaf 40, for control of chicken body louse, 230
Blatta orientalis, 866
Blattella germanica, 211, 239, 573, 608, 866, 867
Blattisocius keegani, 366
Blepharigena spinosula, 562
Blepyrus saccharicola, 92
Blueberries, low-bush, pollination, of 544
Boopedon sp., 465
Bothriocraera bicolor, 92
Boucekiella antoninae, 365
Brachymeria ovata abiesae, 764
Brachyrhinus ligustici, 682
Bracon
brevicornis, 164
xanthonotus, 764
Brevicoryne brassicae, 553, 626
Brevipalpus phoenicis, 636
Bruchidae, 636
Bruchophagus

gibbus, 409
kolobovae, 409
Bruchus brachialis, 401
Brumus suturalis, 92
Bryobia praetiosa, 767
Buccalatrix thurberiella, 296
Bulan, for control of serpentine leaf miner, 358
Butoxy polypropylene glycol, for control of
horn flies, 73, 149
stable flies, 73, 149
Butoxy polypropylene glycol in treatments against biting flies on cattle, 620

Calacoris lineolatus, 636
Calcium arsenate, for control of
bollworm, 42, 646
boll weevil, 42
serpentine leaf miner, 358
Callisto geminatella, 81
Callitroga
hominivorax, 407, 425
macellaria, 407
Capitophorus
braggi, 179
cardiunus, 179
hippophaes, 179
shepheridiae, 179
Captan, in spray combinations against apple insects, 378
Carabid beetles damage Douglas-fir seed, 542
Carbamoyl alkyl phosphorodithioates as chemotherapeutic agents, 445
Carbon disulfide, dosages needed to kill rice weevil, 157
Carpocapsa pomonella, 81, 354, 378, 422, 542, 547, 672, 693
Casinaria sp., 390
Cat-facing in peaches, 27
Cattle, beef
effect of biting fly control on weight gain in, 275
weight responses of in relation to control of flies, 72
Cattle, control of biting flies on, 149
Cattle, dairy, relationship of stable flies to milk production, 269
Cephus cinctus, 804
Cerapterocerus mirabilis, 365
Ceratitus capitata, 234, 679, 921
Ceratocapsus nigellus, injury to peaches, 28
Ceratocystis ulmi, 256
Cerotoma trifurcata, 81
Certacopsis sp., 636
Chaetogaedia sp., near *analis*, 562
Chaetopsis fulvifrons, 81
Chalcids, seed, reared from birdsfoot trefoil, 409
Chalcodermus sp., 636
Cheiloneurus
elegans, 365
sp., 366
Chelonus
heliopae, 164
munakatae, 390
n. sp., 164
Chelycoris sp., 636
Chelymorpha sp., 636
Chermes piceae, 540
Chemotherapeutic agents
phosphorodithioates, carbamoylalkyl, 126
Cheyletia wellsi, 366
Cheyletidae, 366
Chilocorus
angolensis, 92
bivulnerus, 81
spp., 479
Chilo suppressalis, 391, 571
Chipman R-6199, for control of
balsam gall midge, 400
boll weevil, 785
codling moth, 379
desert spider mite, 646
European red mite, 714
red-banded leaf roller, 379
Scolytus multistriatus, 789
two-spotted spider mite, 714
Chipman R-6199
resistance of citrus red mite to, 233
translocation and persistence of in cacao foliage, 773
Chipman R-6200
for control of citrus mealybug, 779
translocation and persistence of in cacao foliage, 775
Chirothrips
falsus, 450
mexicanus, 450
Chlorbenside, for control of European red mite, 713
Chlorbenside, toxicity of to honey bees, 103
Chlordane, for control of
Aceria tulipae, 70
alfalfa snout beetle, 683
bed bug, 242
black carpet beetle, 249
carrot rust fly, 166
cherry fruit flies, 594
chicken body louse, 230
European chafer, 766
horn fly, 795
onion maggot, 884
plum curculio, 132
serpentine leaf miner, 358
sugarcane beetle, 632
tropical rat mite, 413
wireworms, 691
Chlordane
oral chemotherapeutic activity of in mice, 127
resistance of house fly to, 497
Chlordane, toxicity of to
army cutworm, 726
brown-banded cockroach, 610
Ceratitis capitata, 235
melon fly, 236
young cabbage and muskmelon plants, 534
Chlordane-treated soil effective against western subterranean termite, 207
Chlorella pyrenoidosa, 59
Chlorobenzilate, for control of
Aceria tulipae, 70
chicken mite, 911
citrus bud mite, 659
European red mite, 714
mites attacking eye gnat cultures, 462
two-spotted spider mite, 714
Chlorogenus callistephi vulgaris, 512
Chloropicrin, dosages needed to kill rice weevil, 157
Chlorthion, for control of
Aceria tulipae, 70
bed bug, 241
cabbage leaf-feeding lepidoptera, 443
clover leaf weevil, 195
corn earworm, 823
greenbug, 722
green peach aphid, 139
holly bud moth, 88
Mexican bean beetle, 554
Nysius raphanus, 251
onion maggot, 885
serpentine leaf miner, 358
sugarcane beetle, 633
thrips on cabbage, 395
two-spotted spider mite, 736
western tussock moth, 764
Chlorthion
hydrolysis of the alkyl-phosphate bond by rats, cockroaches, and alkali, 800
oral chemotherapeutic activity of in mice, 127
Cholinesterase
activity of bovine red-blood-cell, measurement of, 326
brain, inhibition and recovery of activity in house flies, 750
studies, ET-57 depressed enzyme activity, 121
Choristoneura fumiferana, 264
Chorizagrotis auxiliaris, 726
Chromaphis juglandicola, 427, 527
Chrysodina sp., 636
Chrysomya
megacephala, 147
sp., 147
Chrysopa, 651
Chrysopa
plorabunda group, 366, 613
spp., 90, 713, 757
Chrysoplatycerus splendens, 92
Chrysops univittata, 845
Chymomyza amoena, 734
Cimex
hemipterus, 241
lectularius, 241, 499
Circulifer tenellus, curly top-infective, effect of on watermelon plants, 434
Citrus
mealybugs attacking in California, 90
transpiration of, affected by spray oil, 317
Clepsis peritana, 596
Cnephia pecuarum, 845
Coccinella
californica, 90
novemnotata, 614
transversoguttata, 614
Coccidoxenus sp., 365
Coccophagus
gurneyi, 92
scutellaris, 247
Coccus hesperidum, 480
Coccygomimus sanguinipes, 764
Coeloides dendroctoni, 604
Coelomera sp., 636
Colaspis sp., 636
Coleomegilla
fuscilabris, 81
maculata, 759
octodecimpustuluta, 637
Colias philodice eurytheme, 562
Colladonus clitellarius, 81
Collembola, 252, 767
Collops bipunctata, 613
Compound K-8025, for control of garden tortrix, 597
Compounds
Ammonia-producing, to attract walnut husk fly, 688
biologically active, effect of on house fly ovaries, 384
Compounds
neuropharmacological, ionization and toxicity to insects, 169
neurotoxic toxicity of to the mouse, house fly and cockroach, 171
Compounds, synthetic,
screening tests of 111 for toxicity to house flies, 791

esters of chrysanthemumic acid toxic to house flies, 791
Compounds used in house fly studies,
carbamate, 750
organophosphate, 750
sulfamate, 750
Conocephalus
saltator, 147
strictus, 345
Conotelus mexicanus, 147
Conotrachelus nenuphar, 81, 131, 330, 357
Copper oxychloride sulfate, toxicity of to honey bees, 103
Corn, damage to by southern green stink bug, 471
CP 7769, for control of cotton leafworm, 259
Cotton, Upland, genotypic reaction to boll weevil attack in, 508
Cotton
resistance to boll weevil in mixed populations of resistant and susceptible plants, 502
seedling, Collembola damage to, 252
Crambus calliginosellus damaging carrots, 402
Cremastus chinensis, 390
Crimson clover seed, influence of honey bees on production of, 523
Crioceris duodecimpunctata, 81
Cryolite, for control of
citrus cutworm, 656
garden tortrix, 597
western tussock moth, 764
Cryptolaemus montrouzieri, 90
Culex
gelidus, 109
pipiens, 345
quinquefasciatus, 386, 744
tarsalis, resistance to DDT, 56
tritaeniorhynchus, 109
Culicoides spp., 845
Cultures, bacterial, for control of tobacco insects, 560
Cunaxa sp., 366
Cunaxidae, 771
Cyclocephala borealis, 547
Cyclohexanes, fluorinated, toxicities of to house flies, 413
Cycloneda
munda, 81
sanguinea, 637

Dacus
cucurbitae, 140, 147, 234
dorsalis, 147, 234
Danaus plexippus, attacking sour cherries, 298
DDD, (TDE) for control of codling moth, 542
DDT,
compounds related to evaluated as synergists of, 647
distribution of in soils, 381
DDT, effect on
California red scale, 483
insect predators on alfalfa, 615
parasites of oriental fruit moth, 86
reproduction of house flies, 579
DDT, for control of
alfalfa pests, 730
black carpet beetle, 249
boll weevil, 42, 237, 784
bollworm, 42, 237, 646
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 113, 237
carrot rust fly, 166
chicken body louse, 229
citrus cutworm, 656
codling moth, 357, 378, 422, 542
Colorado potato beetle, 829
corn earworm, 25, 823
Epilachna vigintioctopunctata, 749
European corn borer, 133
Fiorinia externa, 406
garden tortrix, 597
greenbug, 722
holly bud moth, 88
house flies, 20, 809
imported cabbageworm, 254
Japanese beetle, 247
lesser mealworm, 112
Lithocolletis sp., 423
meadow spittlebug, 493
onion maggot, 887
peach tree borer, 558
pink bollworm, 673
purple-backed cabbageworm, 284
Pyroderces rileyi, 24
red-banded leaf roller, 378
rice weevil, 25
root collar borer in yellow-poplar, 252
Scolytus multistriatus, 789
seed-corn maggot, 919
serpentine leaf miner, 358
six-spotted leafhopper, 903
smaller European elm bark beetle, 470
thrips on cabbage, 395
western subterranean termite, 207
western tussock moth, 764
DDT for testing insecticide deposits
with newly hatched codling moth larvae, 547
in bait against confused flour beetle, 301
in oil and in acetone, toxicity to resistant house flies, 914
oral chemotherapeutic activity of in mice, 127
radioactive, in mosquitoes, 640
rates of application to avoid loss of birds and fish, 741
residues of in treated soil, 187
residues of on vegetables, 818
resistance, an attempt to reduce in body lice, 665
susceptibility of to brine shrimp, 780
tests against house fly, 452
use of, causes increase of soft scales on walnut, 427
on vegetables, 307
DDT, resistance of
codling moth to, 693
Colorado potato beetle to, 828
house fly to, 497
potato flea beetle to, 823
stable flies to in Norway, 599
DDT, toxicity of compared with diphenylmethane against the confused flour beetle, 388
DDT, toxicity of to
brown-banded cockroach, 610
buckthorn aphid, 414
foxglove aphid, 414
goldfish, 540
green peach aphid, 414
honey bees, 103
pink bollworm adults, 568
potato aphid, 414
Scolytus multistriatus, 254
DDVP, as a fumigant against house flies, 638
DDVP, for control of
Fannia canicularis, 611
house flies, 20
Mediterranean fruit fly, 679
two-spotted spider mite, 736
DDVP, toxicity of to honey bees, 103
Dehydrogenase systems from fat body of *Blattella germanica*, 867
Demeton, for control of
Aceria tulipae, 70
balsam gall midge, 400
cabbage leaf-feeding lepidoptera, 443
citrus whitefly, 552
corn earworm, 259
European red mite, 255
greenbug, 723
green peach aphid, 139
leaf curl plum aphid, 228
Mexican bean beetle, 259
Nysius raphanus, 251
potato leaf hopper, 259, 336
red-headed pine sawfly, 400
resistant spotted alfalfa aphids, 313
Scolytus multistriatus, 789
serpentine leaf miner, 358
six-spotted leafhopper, 903
soft scales on walnut, 429
thrips, 160, 395
two-spotted spider mite, 711, 714, 736
walnut scale, 528
Demeton
oral chemotherapeutic activity of in mice, 127
residues of on cabbage and celery, 175
translocation and persistence of in cacao foliage, 773
Demeton, resistance to by
citrus red mite, 232
Tetranychus atlanticus, 285
Tetranychus pacificus, 285
Demeton, toxicity of to internal parasites of spotted alfalfa aphid, 375
Dendroctonus
brevicomis, 264
engelmanni, effects of woodpeckers on, 603
Deraecoris
historio, 81
quercicola, injury to peaches, 28
Dermacentor variabilis, 344
Dermanyssus gallinae, 158, 911
Dermatobia hominis, 724
Derris, for control of cherry fruit flies, 594
Desmometopa sp., 147
Diabrotica
speciosa, 636
undecimpunctata howardi, 81
Diachlorus ferrugatus, 845
Dialeurodes citri, 552
Diatraea saccharalis, 163, 562
Diazinon, effect of on
cocooning corn ear moth larvae, 672
reproduction of house flies, 579
Diazinon, for control of
Aceria tulipae, 70
bagworm, 368
bed bug, 241
black carpet beetle, 249
cherry fruit flies, 595
chicken mite, 911
clover leaf weevil, 196
codling moth, 422
corn earworm, 259
desert spider mite, 646
garden tortrix, 597
Fannia canicularis, 611
holly bud moth, 88
horn flies, 559
house flies, 20, 908
leaf curl plum aphid, 228
Mexican bean beetle, 259, 554
Nysius raphanus, 251
onion maggot, 885
potato leafhopper, 259
serpentine leaf miner, 358

western subterranean termite, 209
western tussock moth, 764
Diazinon
hydrolysis of the alkyl-phosphate bond by rats, cockroaches, and alkali, 800
oral chemotherapeutic activity of in mice, 127
Diazinon, resistance to of
citrus red mite, 233
house fly to, 497, 546, 616
Dibrachys cavus, 764
Dibrom for control of
iris borer, 550
two-spotted spider mite, 736
Dicapthon (see also Am. Cyanamid 4124) for control of
chicken mite, 911
citrus cutworm, 656
western tussock moth, 764
Dicapthon
hydrolysis of the alkyl-phosphate bond by rats, cockroaches, and alkali, 800
resistance of house fly to, 497
Dichelops melacanthus, 636
Dieldrin
as a seed treatment against wireworms, 294
as a permanent mothproofing agent, 887
conversion of aldrin to in soil, 1
detection of by potato flea beetle, 825
dips to reduce weevil damage to pine seedlings, 916
effect on insect predators on alfalfa, 615
from converted aldrin in carrots, 340
susceptibility of to brine shrimp, 780
Dieldrin, for control of
Aceria tulipae, 70
alfalfa snout beetle, 682
bagworm, 368
bed bug, 241
black carpet beetle, 249
boll weevil, 237, 490
bollworm, 238, 490
cabbage leaf-feeding lepidoptera, 443
carrot rust fly, 166
clover leaf weevil, 196
Colorado potato beetle, 829
cotton aphids, 238
cotton fleahopper, 490
European chafer, 766
Fuller rose beetle, 284
meadow spittlebug, 493
northern fowl mite, 188
Nysius raphanus, 251
onion maggot, 884
pink bollworm, 238
plum curculio, 131
rice paddy borer, 571
seed-corn maggot, 919
serpentine leaf miner, 358
sheep ked in Oregon, 585
smaller European elm bark beetle, 470
sugarcane beetle, 631
thrips, 395, 489
two-spotted mite, 238
weevils on crimson clover, 459
wireworms, 691
Dieldrin
oral chemotherapeutic activity of in mice, 127
percentage recovered from treated soils, 201
persistence and transformation of in soils, 214
residues in treated soil, 187
residues of on alfalfa, 392
resistance of house fly to, 497
Dieldrin, toxicity of to
army cutworm, 726
boll weevil, 670
Ceratitis capitata, 235
pink bollworm adults, 568
Scolytus multistriatus, 257
young cabbage and muskmelon plants, 534
Dieldrin-treated soil, effect of on
adults of European chafer, 486
western subterranean termite, 207
Diethyltoluamide
in treatments against biting flies on cattle, 620
ultraviolet determination of, 694
Diets
media for pink bollworm, 309
larval, for rearing the boll weevil, 288
Digamasellus angulosus, 770
Dilan, for control of
alfalfa snout beetle, 683
citrus cutworm, 656
garden tortrix, 597
serpentine leaf miner, 358
western tussock moth, 764
Dilan, toxicity of in soil to *Ceratitis capitata*, 235
Dimefox biological and chemical oxidation of, 49
Dimefox for control of
balsam hall midge, 400
citrus mealybug, 779
Scolytus multistriatus, 789
Dimefox, not oxidized in *Chlorella* cultures, 59
Dimite, for control of mites attacking eye gnat cultures, 462
Dimethoate (see Am. Cyanamid 12880)
Dinitrobutylphenol, for control of leaf curl plum aphid, 228
Dinitrocresol, for control of European red mite, 713
Dinitro phenol, for control of leaf curl plum aphid, 228
Dinitro 289, for control of soft scales on walnut, 430
Diphacinone, ineffective against confused flour beetle, 302
Diphenylmethane, derivatives, relation of structure to toxicity to confused flour beetle, 385
Dips, preplanting, reduce weevil damage to pine seedlings, 915
Dipterex, for control of
bed bug, 241
boll weevil, 42, 784
bollworm, 42
cabbage leaf-feeding lepidoptera, 443
codling moth, 422
house flies, 20, 453, 908
Mexican bean beetle, 554
onion maggot, 885
serpentine leaf miner, 358
thrips on cabbage, 395
two-spotted spider mite, 736
western tussock moth, 764
Dipterex
oral chemotherapeutic activity of in mice, 127
resistance of house fly to, 497, 616
toxicity of to honey bees, 103
Disonycha pennsylvanica, 81
Dissosteira carolina, 345
DNBP, for control of
apple grain aphid, 355
rosy apple aphid, 355
DN-40, for control of
apple grain aphid, 355
rosy apple aphid, 355
Douglas-fir seed, damage to by carabid beetles, 542
Dowco 109, for control of
cattle grubs, 583, 876
cattle lice, 876
Dow ET-15, for control of
boll weevil, 42
bollworm, 42
house flies, 20
Dow ET-15, toxicity of to honey bees, 102
Dow ET-57 as a fumigant against
American cockroach, 409
confused flour beetle, 409
house fly, 409, 638
yellow-fever mosquito, 409
Dow ET-57, for control of
bed bug, 241
cattle grubs, 119, 545, 583, 783, 876
cattle lice, 867
chicken body louse, 189, 230
chicken shaft louse, 189, 559
fluff louse, 189
house fly, 361, 908
horn flies, 559
lone star tick, 425
northern fowl mite, 189
onion maggot, 884
screw-worm, 425
serpentine leaf miner, 358
stable fly, 425
sticktight flea, 189
wing louse, 189
Dow ET-57
effect of treatment time on control of cattle grubs, 808
fumigation properties of, 408
hydrolysis of the alkylphosphate bond by rats, cockroaches, and alkali, 800
not oxidized in *Chlorella* cultures, 59
oral chemotherapeutic activity of in mice, 127
resistance of house fly to, 497
toxicity of to honey bees, 103
Draeculacephala mollipes, 81
Drepanaphis granovskyi, 179
Drosophila
affinis, 733
busckii, 733
duncani, 734
funebris, 733
immigrans, 733
melanogaster, 12, 81, 179, 222, 337, 685, 733
putrida, 733
quinaria, 733
repleta, 734
robusta, 733
tripunctata, 733
transversa, 734
Dysdercus pallidus, 633
Dysinicoccus obesus, 555

Echidnophaga gallinacea, 188
Ectoparasites on Alabama mammals, 260
Edessa
meditabunda, 637
sp., 636
Empoasca fabae, 220, 258, 336, 729
Empusa
aphidis, 185
no sp., 184
sphaerosperma, 185
Endrin, as a seed treatment against wireworms, 294
Endrin, effect of
climatic factors on toxicity of to boll weevil, 670

on cocooning codling moth larvae, 672
Endrin, for control of
Aceria tulipae, 70
alfalfa snout beetle, 683
boll weevil, 42, 95, 784
bollworm, 42, 237, 646
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 237
clover leaf weevil, 195
corn earworm, 25
cotton leafworm, 237, 259, 784
cyclamen mite, 431
Epilachna vigintioctopunctata, 749
European chafer, 766
meadow spittlebug, 493
Nysius raphanus, 251
onion maggot, 884
paddy borer, 411, 571
pink bollworm, 673
Pyroderces rileyi, 25
rice weevil, 25
seed-corn maggot, 919
serpentine leaf miner, 358
salt-marsh caterpillar, 237
sugarcane borer, 562
thrips on cabbage, 395
Endrin, residues of on vegetables, 818
Endrin, toxicity of to
army cutworm, 726
Ceratitis capitata, 235
melon fly, 236
pink bollworm adults, 578
young cabbage and muskmelon plants, 534
Enoplopactus heterothorax, 636
Entomobrya unostrigata, 252
Entomologist, or forester, 264
Entomophagus species, role of in natural control of insect populations, 474
Entomophthora
exitialis, 341
oronata, 341
virulenta, 341
ENT 21478, as a lure for Mediterranean fruit fly, 679
Eomenacanthus stramineus, 188
Ephestia
elutella, 417
figulilella, 596
Epilachna
varivestis, 258, 345, 553, 652, 735
vigintioctopunctata, 749
Epitrix cucumeris, feeding behavior, 823
EPN, effect of on parasites of oriental fruit moth, 83
EPN, for control of
boll weevil, 42, 784
bollworm, 42
corn earworm, 823
cyclamen mite, 432
house flies, 20, 908
plum curculio, 132
serpentine leaf miner, 358
wireworms, 691
EPN, resistance of citrus red mite to, 233
Equipment
automation system for insectaries, 109
black-light traps used to attract hornworm moths, 79
broadcaster for applying granulated insecticides, 278
device for rapid application of measured volumes of toxicants, 261
device to evaluate repellent effect of diphenylmethane, 389
drop net device to determine mosquito prevalence, 321
for irradiation studies, 575
for recording temperatures and humidity of microenvironment, 397
modified mechanical counter, 112
EQ-335, for control of screw-worms, 408
Ereynetes sp., 366
Eriopis conveza, 637
Eristalis arvorum, 147
Erycideus sp., 636
Erythraeus sp., 366
Erythrothrips arizonae, 450
Estigmene acrea, 237, 296, 562
Ethion, for control of
apple grain aphid, 355
codling moth, 357, 379, 422, 542
desert spider mite, 646
European red mite, 355, 422
greenbug, 722
onion maggot, 885
red-banded leaf roller, 379
rosy apple aphid, 355
serpentine leaf miner, 358
soft scales on walnut, 430
two-spotted spider mite, 356, 710, 714, 736
walnut scale, 530
Ethylene chlorobromide, relation between load and concentration of fumigant, 896
Ethylene dibromide
dosages needed to kill rice weevil, 157
relation between load and concentration of fumigant, 896
Ethylene dichloride
dosages needed to kill rice weevil, 157
emulsion, for control of, peach tree borer, 558
Eucelatoria armigera, 562
Euetheola rugiceps, 631
Euschistus tristigmus, 81
Euxesta quadrivitatta, 147
Euzophora ostricolorella, 251
Evergestis
pallidata, 281
rimosalis, 254
Exeristes roborator, 652
Exochomus flavipes, 92

Fabric pests, feeding of on natural and synthetic textiles, 248
Fannia
canicularis, 611
spp., 599
Fenson, for control of European red mite, 713
Fenusa pusilla, 245
Flavor evaluations of cacao from trees treated with systemic insecticides, 778
Fleas, on antelope ground squirrel, 32
Fleck, chlorotic, an eriophyid mite injury of plum, 335
Flies, tachinid, reared from lepidopterous larvae in Arizona, 561
Folidol, for control of paddy borer, 411, 571
Forester, or entomologist, 264
Foxella ignota utahensis, 33
Frankliniella
exigua, 394, 450
fusca, 115, 161, 394
gossypiana, 450
insularis, 450
minuta, 450
occidentalis, 450
paucispinosa, 636
sp., 489, 872
tenuicornis, 394
tritici, 115, 161, 394, 451
Fumigants, susceptibility of stages of rice weevil to, 157
Fumigation
effects of methyl bromide on viability of seeds of grain, 847
food commodities for insect control, factors affecting, 891
laboratory tests of organic compounds, 638
methyl bromide against European chafer, 869
methyl bromide, fruit under plastic tarpaulins, 436
warehouse, flue-cured tobacco with HCN against cigarette beetle, 417
Fungi, entomogenous, to control aphids on potato in Maine, 184

Galerucella
notulata, 81
xanthomelaena, 296
Gargaphia torresi, 636
Garmania bulbicola, 366
Gelis tenellus, 650
General Chemical Compound 3707, for control of, house flies, 908
Genite, for control of European red mite, 713
Geocoris
decoratus, 613, 634
punctipes, 757
ventralis, 637
Glycine-sodium hydroxide solution, an attractant for walnut husk fly, 532, 687
Glyodin, in spray combinations against apple insects, 378
Goldfish, toxicity of Sevin to, 540
Goniocotes gallinae, 188
Graphocephala versuta, 81
Grapholitha molesta, 81, 82
Gulfspray naphtha, for control of black carpet beetle, 249
Gummosis in peaches, 27
Guthion, effectiveness against California red scale, 912
Guthion, effect of climatic factors on toxicity of to
boll weevil, 670
desert spider mite, 670
Guthion, effect of on cocooning codling moth larvae, 672
Guthion, for control
boll weevil, 42, 238, 490, 784
bollworm, 42, 238, 490, 646
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 254
codling moth, 379, 422
cotton aphid, 238
cotton fleahopper, 490
cotton leafworm, 259, 784
cross-striped cabbageworm, 254
desert spider mite, 646
European red mite, 422, 714
green peach aphid, 139
house flies, 908
imported cabbageworm, 254
Lithocolletis sp., 423
onion maggot, 885
paddy borer, 411
pink bollworm, 238, 673
red-banded leaf roller, 379
serpentine leaf miner, 358
soft scales on walnut, 430
sugarcane beetle, 633
thrips, 395, 489
two-spotted spider mite, 238, 714, 736
walnut scale, 530
western tussock moth, 764
Guthion, half-life of on cotton leaves, 831

Guthion, toxicity of to
honey bees, 103
pink bollworm adults, 568
Gypona octolineata, 81

Haemagogus equinus, 744
Haematopinus suis, 255
Haplothrips
fasciculatus, 451
purpuratus, 451
Harpalus cautus, 543
HCN, for control of
cigarette beetle, 417
tobacco moth, 417
Health hazards involved in the use of organic phosphorus insecticides, 831
Heat treatment to eliminate European chafer, 912
Heliothis
gelotopoen, 635
spp., 560, 562, 633
virescens, 635, 560
zea, 24, 41, 236, 258, 296, 503, 560, 637, 645, 735, 783, 822
Heliothis zea, effect of fertilizer applications on populations of, 757
Heliothrips haemorrhoidalis, 386
Hemerocampa vetusta, 762
Hemiberlesia
lataniae, 114
rapax, 114
Heptachlor, as a
fumigant against house flies, 638
seed treatment against wireworms, 294
Heptachlor, conversion to epoxide,
in soil, 1
on plants, 3
Heptachlor dips to reduce weevil damage to pine seedlings, 916
Heptachlor, for control of
alfalfa snout beetle, 682
boll weevil, 42, 114, 784
bollworm, 42
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 113
carrot rust fly, 167
clover leaf weevil, 195
clover root curculio, 555
Colorado potato beetle, 829
corn earworm, 823
European chafer, 766
Nysius raphanus, 251
onion maggot, 884
plum curculio, 131
seed-corn maggot, 919
serpentine leaf miner, 358
sugarcane beetle, 631
thrips on cabbage, 395
weevils on crimson clover, 459
western subterranean termite, 209
wireworms, 464, 691
Heptachlor
metabolism of by house flies, 346
percentage recovered from treated soils, 202
persistence of in soils, 11
residues on alfalfa, 222, 392
Heptachlor, toxicity of to
army cutworm, 726
brown-banded cockroach, 610
pink bollworm adults, 578
Scolytus multistriatus, 257
cabbage and muskmelon plants, 534
Hercothrips
braziliensis, 636
marginipennis, 451
Hercules AC-426, for control of chicken body louse, 230
Hercules AC-528, effectiveness against California red scale, 912
Hercules AC-528, for control of
codling moth, 422
corn earworm, 823
cotton leafworm, 259
desert spider mite, 646
European red mite, 714
lone star tick, 425
Mexican bean beetle, 554
mites attacking eye gnat cultures, 462
onion maggot, 885
screw-worm, 425
stable fly, 425
two-spotted spider mite, 714, 736
western tussock moth, 764
Hercules AC-528
resistance of citrus red mite to, 233
toxicity of to honey bees, 103
Heterobostrychus aequalis, 556
Heterothrips prospopidis, 451
Hexamethylphosphoramide, distribution of radioactive in the cockroach, 49
Hibernation, survival and emergence of boll weevils, 745
Hippelates, 278, 557
bishoppi, 557
collusor, 461
pusio, 557
Hippiscus rugosus, 345
Hippodamia
convergens, 375, 612, 722, 759
parenthesis, 614
sinuata disjuncta, 613
quinquesignata, 375
Holcomb C-326, toxicity to honey bees, 103
Honey bee (see *Apis mellifera*) influence of on production
of crimson clover seed, 523
substitute sugar source for, 287
wintering in Minnesota, 332
Hoplopsyllus anomalus, 33
Horcias nobilellus, 636
Horistonotus, 634
Hyalopterus
atriplicis, 179
pruni, 227
Hydrocyanic acid, dosages needed to kill rice weevil, 157
Hydrogen cyanide, relation between load and concentration of fumigant, 896
Hydrogen phosphide, as a fumigant against grain and flour insects, 900
Hydrolysate, yeast, influence of on melon fly, 140
Hylastinus obscurus, 491
Hylemya
antiqua, 883
brassicae, 534
cilicrura, 690, 704, 919
Hylobius pales, 915
Hylurgopinus rufipes, 789
Hypera
brunneipennis, 296
meles, 459
nigrirostris, 459
postica, 392, 506
punctata, 195
Hyperaspis, 614
festiva, 634, 637
jucunda, 92
lateralis, 90
punctata, 366
sp., near *globula*, 92
sp., 770
Hypoderma
bovis, 359, 445, 545, 582, 793, 808, 876
lineatum, 359, 445, 545, 582, 793, 808, 876
spp., 119

Ichneumonidae, characteristics of in relation to biological control, 650
IN-930 as synergist for pyrethrins, 742
Insects
associated with passion fruit flowers in Hawaii, 147
attracted to light traps, 550
control of, affecting birdsfoot trefoil seed production, 492
control programs, function of fish and Wildlife Service in, 740
effectiveness of Dow ET-57 as a fumigant against, 409
Insect
evaluation of plastic coated glass fiber screens, 864
predators, effect of insecticides on alfalfa, 615
role of weather and entomophagus species in natural control of, 474
survival in jet aircraft, 239
thermal death points of, 344
transient, in Delaware's apple and peach plantings, 81
Insecticides
against paddy borer in Taiwan, 571
broadcaster for applying granules, 278
changes in common names of, 373
decline of in soil, 198
dialkyl aryl phosphorothioate, hydrolysis of the alkylphosphate bond by rats, cockroaches, and alkali, 800
effect of certain organic on mortality and oviposition of pink bollworm, 567
effect of climatic factors on toxicities of, 669
effect of on codling moth larvae seeking cocooning quarters, 672
effect of on hatchability and taste, 190
effect of seed treatments of cotton with systemic, 697
effect of soil drenches on young cabbage and muskmelon plants, 534
effect of temperature on against boll weevil, 93
factors influencing recovery and transformation of in soil, 214
granulated, for control of carrot rust fly, 167
for control of insects on snap beans, 258
granulated for control of weevil on crimson clover, 459
granulated for wireworm control, 463
granulated, systemics against spotted alfalfa aphid, 122
granules of heptachlor against boll weevil, 114
fumigation tests of organic compounds, 638
induce increase of soft scales on walnut, 427
insect predators collected on alfalfa before and after treatment with, 615
movement of in soils, 380
multiple regression to compare results of control of meadow spittlebug, 743
new, for control of codling moth, 422
organic, effect of on parasites of oriental fruit moth, 82
organophosphate, developed on rational principles, 714
organophosphorus, metabolism of by microorganisms, 59

organic phosphorus, public health hazards involved in the use of, 831
organophosphorus, resistance of house flies to, 616
residual effect of for control of European chafer, 765
residual sprays on dairy farms against house flies, 908
residual toxicity of to smaller European bark beetle, 256
residues of Diazinon on cherries, 595
residues of on alfalfa, 392
residues of on vegetables, 818
residues of Thimet in small grains grown in treated soil, 623
seed treatments against wireworms, 690
soil, against plum curculio, 131
stable fly resistance to DDT in Norway, 599
susceptibility of brown-banded cockroach to, 608
susceptibilities of four species of potato-infesting aphids to, 414
systemic, action on bloodsucking ectoparasites of 28 compounds given orally to rabbits, 499
systemic, against green peach aphid, 138
systemic, effect of seed treatments on cotton, 495
systemic, field tests with for control of cattle grubs, 793
systemic, for control of cattle grubs, 582
systemic, for control of *Scolytus multistriatus*, 789
systemic, for protection against spotted alfalfa aphid, 457
systemic, for *Theobroma cacao*, 773
systemic, translocation and persistence of in cacao foliage, 773
systemic, trunk implantation in conifers, 400
Thimet granules against leaf miners, 245
timing applications to control European corn borer, 133
Ionization and insect toxicity, 169
Iridomyrmex humilis, 147, 480
Irradiated wheat, effects of on stored grain insects, 674
Irradiation, visible spectrum, effect of on growth in insects, 573
Iseropus orgyiae, 764
Isobornyl thiocyanoacetate, toxicity of in soil to *Ceratitis capitata*, 235
Isodrin, for control of
alfalfa snout beetle, 683
European chafer, 766
onion maggot, 884
serpentine leaf miner, 358
Isolan, translocation and persistence of in cacao foliage, 773
Isomerism, *cis-trans*, effect of on potency of an insect attractant, 921
Iso-Systox, resistance of citrus red mite to, 233
Isovaleryindandione, effect on confused flour beetle, 302
Itonida balsamicola, 400
Itonididae, 366
Itoplectis conquisitor, 650

Jadera sanguinolenta, 635

K.A.A.D. mixture, 740
Karathane, for control of *Aceria tulipae*, 70
Karathane, toxicity of to honey bees, 103
Kelthane, for control of
chicken mite, 911
desert spider mite, 646
European red mite, 255, 714
two-spotted spider mite, 714, 736
Kelthane, resistance to by
Tetranychus atlanticus, 286
Tetranychus pacificus, 286
Kepone, for control of onion maggot, 887
Kerosene, effectiveness against California red scale, 912
Khapra beetle, food habits of larva, 465
Kurtomathrips morrilli, 451

Labauchena daguerrei, 112
Labidura riparia, 366
Lamprophorus tenebrosus, 390
Laphygma
exigua, 296, 562
frugiperda, 562, 634
Lasioderma serricorne, 417
Lasioseius penicilliger, 770
Lasius alienus americanus, 555
Lead arsenate, for control of
bagworm, 368
cherry fruit flies, 594
codling moth, 357, 379, 542
red-banded leaf roller, 379
serpentine leaf miner, 358
Lecanium
cerasorum, 427, 527
corni, 427
pruinosum, 427
Lecopis bella, 90
Ledermuelleria n. sp., 770
Leioseius minusculus, 767
Lepidosaphes beckii, 476
Leptinotarsa decemlineata, 345, 828
Leptocoriza acuta, 405
Leptomastidea abnormis, 92
Leptomastix dactylopii, 92
Leptoterna dolabratus, injury to peaches, 28
Leptothrips
sp. near *mali*, 451
sp., 636
Lerodea eufala, 562
Lethane 384, for control of
black carpet beetle, 249
horn flies, 73, 154
stable flies, 73, 154
Lethane 384 in treatments against biting flies on cattle, 620
Lettuce-yellows prevention, with reference to aster-yellows virus infection and transmission by *Macrosteles fascifrons*, 512
Leucocytozoon disease, effect of black fly control on the incidence of, 845
Leucophenga varia, 733
Light, black, response of hornworm moths to, 78
Light traps, insects attracted to, 550
Limonius agonus, 690
Lindane, as a
fumigant against house flies, 638
seed treatment against wireworms, 294
Lindane, for control of
Aceria tulipae, 70
alfalfa snout beetle, 683
bed bug, 242
black carpet beetle, 249
body lice, 742
carrot rust fly, 166
Ceratitis capitata, 235
chicken body louse, 229
clover leaf weevil, 195
cyclamen mite, 432
horn flies, 559
house flies, 908
melon fly, 236
plum curculio, 132
Pagiocerus fiorii, 468
Scolytus multistriatus, 257
serpentine leaf miner, 358
tropical rat mite, 413
western subterranean termite, 207
wireworms, 691
Lindane
effectiveness of for protection of stored wheat, 843
effect of butter fat content of milk on toxicity to adult house flies, 561
movement of in soils, 381
oral chemotherapeutic activity of in mice, 127
relative toxicity of brown-banded cockroach to, 610
residues of in milling fractions from treated wheat, 516
residues of on vegetables, 818
resistance of house fly to, 497
to prevent emergence of smaller European elm bark beetle, 470
Lindane, toxicity of to
buckthorn aphid, 414
foxglove aphid, 414
green peach aphid, 414
potato aphid, 414
Therioaphis maculata and *Hippodamia*, 377
Lindorus lophanthae, 479
Linognathus vituli, 877
Lipeurus caponis, 188
Liriomyza pusilla, 357
Liroaspes sp., 366
Listronotus oregonensis, 37
Lithocolletis sp., 423
Lixophaga diatraea, 163
Lobodiplosis sp., 366
Loxostege similalis, 562
Lures, see baits
Lyctocoris campestris, 764
Lygus
lineolaris, 28, 81, 493, 729
pallidulus, 636
Lysiphelbus spp., 375

Macrocentrus
ancylivorus, 85
delicatus, 86
Macronoctua onusta, 549
Macrosiphum
albifrons, 179
creelii, 179
granarium, 178, 553
nigromaculosum, 179
pisi, 169, 178, 613
solanifolii, 144, 184, 414
Macrosteles fascifrons, 512, 903
Mansonia perturbans, 320
Marietta
graminicola, 363
mexicana, 115
picta, 365
Malathion, effect of on soft scales on walnut, 429
Malathion, for control of
Aceria tulipae, 70
bagworm, 368
bed bug, 241
black carpet beetle, 249
body lice, 742
boll weevil, 42, 96, 490, 784
bollworm, 42, 490
cabbage leaf-feeding lepidoptera, 443
California red scale, 257
cherry fruit flies, 594
chicken body louse, 229

chicken mites, 158
citrus cutworm, 656
citrus red mite, 257
citrus whitefly, 552
clover leaf weevil, 196
codling moth, 379
corn earworm, 259, 823
cotton fleahopper, 490
cotton leafworm, 259, 784
cyclamen mite, 432
Epilachna vigintioctopunctata, 749
Fannia canicularis, 611
Fiorinia externa, 406
garden tortrix, 597
hog louse, 255
holly bud moth, 88
honey bees, 108
horn flies, 559
house fly, 361, 908
imported cabbageworm, 254
leaf curl plum aphid, 228
lesser mealworm, 112
loblolly mealybug, 556
Mexican bean beetle, 259, 554
northern fowl mite, 158
Nysius raphanus, 251
onion maggot, 885
Pagiocerus fiorii, 468
potato leafhopper, 259
red-banded leafroller, 379
resistant spotted alfalfa aphids, 313
serpentine leaf miner, 358
six-spotted leafhopper, 903
smaller European elm bark beetle, 470
thrips, 395, 489
two-spotted spider mite, 736
walnut scale, 528
western subterranean termite, 209
western tussock moth, 764
Malathion
detection of by potato flea beetle, 825
effective for protection of stored wheat, 843
ineffective against variegated cutworm, 410
oral chemotherapeutic activity of in mice, 127
suspected cases of poisoning in the Mississippi Delta area, 832
Malathion, residues of on
alfalfa, 392
milling fractions from treated wheat, 516
turnip tops, collards, snap beans, and lettuce, 307
vegetables, 818
Malathion, resistance to by
citrus red mite, 233
house flies, 18, 452, 497, 546, 616
Malathion, toxicity of to
boll weevil, 670
buckthorn aphid, 414
Ceratitis capitata, 235
foxglove aphid, 414
green peach aphid, 414
melon fly, 236
internal parasites of spotted alfalfa aphid, 375
pink bollworm adults, 578
potato aphid, 414
Scolytus multistriatus, 257
Malathion, toxicity and anticholinesterase activity compared with acethion, 716
Medetera aldrichii, 604
Megachile sp., 466
Melanoplus
femur-rubrum, 345
spp., 866
Melanotus
communis, 291, 463
sp., 690
Melcha ornatipennis, 390
Melophagus ovinus, 585
Menacanthus stramineus, 229
Menopon gallinae, 188, 558
Mesostigmata, 259, 768
Metacide, effect of on parasites of oriental fruit moth, 83
Metacide for control of
cyclamen mite, 431
serpentine leaf miner, 358
Metagonistylum minense, 163
Metaphycus
helvolus, 247
lounsburyi, 247
luteolus, 274
stanleyi, 247
Methods
application of insecticides against carrot rust fly, 165
artificial dissemination of fungi against spotted alfalfa aphid, 341
automation system for insectaries, 109
colorimetric whole-blood, to measure cholinesterase activity, 326
determining persistence of heptachlor in soils, 12
evaluating insecticide-treated soils against termites, 207
evaluating sweetclover weevils' preference for sweetclover plants, 190
field studies of the balsam woolly aphid, 540
for proving conversion of, heptachlor to its epoxide on plants, 3; aldrin to dieldrin on plants, 8
for studying biology of carrot weevil, 37
for studying decline of soil insecticides, 199
killing and fixing lepidopterous larvae, 740
polyethylene cover to suppress house fly breeding, 411
probit analysis on a digital computer, 738
radioactive tracer, to study responses of wireworms to chemical seed treatments, 291
rapid determination of sex in walnut husk fly, 551
rapid rearing of clothes moth eggs, 919
rearing carpenterworms, 406
rearing clover root curculio larvae for research uses, 917
rearing large colonies of *Aceria tulipae* in pure culture, 303
sampling to determine prevalence of *Mansonia perturbans*, 320
screening and evaluating compounds for chemotherapeutic activity, 126
self-applicating devices in horn fly control, 795
studying absorption and metabolism of heptachlor by house flies, 346
studying microenvironment of the spotted alfalfa aphid, 397
synthesis of carboxy-labeled C^{14}-*N,N*-diethyl-*m*-toluamide, 707
timing of applications against European corn borer, 133
treadle sprayer and cable-type back rubber to control biting flies, 149
ultraviolet determination of diethyltoluamide, 694
use of brine shrimp for bioassay, 780
use of vermiculite in medium for stable fly larvae, 250
wintering honey bees in Minnesota, 332
Methoxychlor, for control of
bed bug, 242
biting flies on cattle, 618
black carpet beetle, 249
cabbage leaf-feeding lepidoptera, 443
cherry fruit flies, 594
chicken body louse, 230
clover leaf weevil, 196
codling moth, 542
holly bud moth, 88
horn fly, 149, 276, 559, 746, 795
house flies, 908
imported cabbageworm, 254
Pagiocerus fiorii, 468
serpentine leaf miner, 358
stable fly, 149, 276
thrips on cabbage, 395
tropical rat mite, 413
Methoxychlor
oral chemotherapeutic activity of in mice, 127
residues of in milling fractions from treated wheat, 516
residues of on vegetables, 818
resistance of house fly to, 497
Methyl bromide
dosages needed to kill rice weevil, 157
effect of different food commodities on concentration of fumigant, 895
effects of fumigation on viability of seeds of grain, 847
fumigation against European chafer, 869
fumigation of fruit under plastic tarpaulins, 436
Methyl chlorobenzilate, toxicity of to honey bees, 103
Methyl demeton, for control of
Aceria tulipae, 70
serpentine leaf miner, 359
Methyl demeton
resistance of citrus red mite to, 233
translocation and persistence of in cacao foliage, 773
Methyl parathion, for control of
boll weevil, 42, 490, 670, 784
bollworm, 42, 490
cabbage looper, 113
cotton fleahopper, 490
cotton leafworm, 784
desert spider mite, 671
garden tortrix, 597
greenbug, 722
Nysius raphanus, 251
onion maggot, 885
paddy borer, 411
thrips, 489
western tussock moth, 764
Methyl parathion
half-life of on cotton leaves, 831
hydrolysis of the alkylphosphate bond by rats, cockroaches, and alkali, 800
Metopius, 652
Metriona bicolor, 81
MGK-264, for control of
horn flies, 73, 276
stable flies, 73, 270, 276
Microcephalothrips abdominalis, 451
Microgaster russata, 390
Milichiella lacteipennis, 147
Mites
parasitic on Alabama mammals, 260
soil-inhabiting from Connecticut apple orchards, 767
Mocis sp., 636
Moisture, effect of on development of weevils and fungi in wheat, 701

Molecular configuration, effect of on toxicity to house flies, 181
Monarthropalpus buxi, 245
Monecphora bicincta, 81
Monocrepidius vespertinus, 81
Monohydrated copper sulfate, toxicity of to honey bees, 103
Monsanto CP-7769, for control of
boll weevil, 490, 785
bollworm, 490
cotton fleahopper, 490
desert spider mite, 646
thrips, 490
Monsanto 8574, for control of two-spotted spider mite, 736
Monsanto 10502, for control of two-spotted spider mite, 736
Monsanto 10851, for control of two-spotted spider mite, 736
Monsanto 11327, for control of two-spotted spider mite, 736
Monsanto 11448, for control of two-spotted spider mite, 736
Moranila californica, 247
Musca domestica, 1, 18, 39, 43, 76, 169, 181, 211, 239, 250, 345, 346, 361, 384, 386, 409, 411, 413, 452, 546, 559, 560, 579, 599, 612, 616, 638, 647, 666, 675, 715, 742, 746; 750, 791, 798, 810, 839, 880, 908, 914
Muscina stabulans, 612
Myzocallus pulchellus, 179
Myzus
monardae, 179
persicae, 138, 145, 178, 414, 812
solani, 145, 414

Nabam in seed treatments of cotton, 697
Nabis
alternatus, 613
spp., 757
Narlene, for control of human bot fly, 724
Naupactus sp., 636
Neisthrea pictipes, 636
Nemorilla floralis, 562
Neoclytus acuminatus, 81
Neodiprion
lecontei, 400
nigroscutum, 125
swainei, 125
Neolygus, damage to peaches, 28
canadensis, 28
caryae, 29
omnivagus, 29
geneseensis, 28
quercalbae, 29
Neotran, persistence of residues, 914
Nephus sp., 92
Netelia, 652
Nezara viridula, 471
Niagara 908, for control of two-spotted spider mite, 736
Niagara 1240 (see Nialate)
Nialate, for control of leaf curl plum aphid, 229
Nicotine, for control of
leaf curl plum aphid, 228
serpentine leaf miner, 358
walnut scale, 528
Nicotine, toxicity of to
Ceratitis capitata, 235
Therioaphis maculata and *Hippodamia*, 377
N,N-diethyl-*m*-toluamide, an insect repellent, 707
N,N-diethyl-*m*-toluamide, for control of horn flies, 276
stable flies, 276
Norvellina seminuda, 81
Nosema disease, 46
NPD, for control of
Aceria tulipae, 70
serpentine leaf miner, 358
Nutrition, comparative NPK, of mites on apple trees, 369
Nysius
raphanus, 250
stimulans, 636

Obituary
Orlando S. Bare, 565
Dwight Fletcher Barnes, 117
Joseph M. Davis, 415
Henry James Franklin, 564
John V. Schaffner, Jr., 266
Harry Scott Smith, 266
Mathis Charles Tanner, 117
Oils, angelica, for trapping Mediterranean fruit flies, 681
Oils, California spray, for control of
California red scale, 193
citrus red mite, 193
Oil, dormant, for control of European red mite, 713
Oil, effect of on transpiration of citrus, 317
Oil emulsion, for control of
citrus whitefly, 552
soft scales on walnut, 427, 429
Oils, for control of
leaf curl plum aphid, 228
rufous scale, 404
Oil, miscible, for control of European red mite, 355
Oil, petroleum, for control of citrus bud mite, 659
Oil-base sprays, for control of black carpet beetle, 249
Olla n. sp., 637
Ololaelaps
hemisphaera, 771
placentula, 771
Oncometopia undata, 81
Oncopeltis fasciatus, 169, 573
Ophionini, 652
Ophyra nigra, 147
Orasema sp., 112
Orchopeas leucopus, 33
Oribatei, 769
Orius
insidiosus, 390, 757
sp., 637
tristicolor, 613
Ornithonyssus
bacoti, 413
sylviarum, 158, 188
Orthopodomyia
alba, 744
signifera, 744
Orthorix, for control of *Aceria tulipae*, 70
Oryzaephilus surinamensis, 674, 900
Otosema odorata, 637
Ovex, for control of
European red mite, 355, 714
mites, attacking eye gnat cultures, 462
two-spotted spider mite, 714
Oxycarenus hyalipennis, 633

Pachylobius picivorus, 915
Pagiocerus fiorii, 468
Panonychus
citri, 193, 232, 353, 914
ulmi, 255, 263, 352, 356, 369, 422, 527, 713
Pantomorus
godmani, 284
sp., 636
Paracalocoris scrupeus, injury to peaches, 28
Paraphaenodiscus sp., 366
Paraphlepsius irroratus, 81
Parascaptomyza disticha, 733
Parasites
attempts to establish against sugarcane borer in La., 163
effect of organic insecticides on, 82
release of in California against citrus mealybugs, 90
species of *Thysanus*, 114
Parasitoidea, 767
Parasitus americanus, 767
Parathion, effect of on
cocooning codling moth larvae, 672
insect predators on alfalfa, 615
parasites of oriental fruit moth, 85
soft scales on walnut, 429
Parathion, for control of
Aceria tulipae, 70
alfalfa pests, 730
alfalfa snout beetle, 683
bagworm, 368
bollworm, 646
cabbage leaf-feeding lepidoptera, 443
carrot rust fly, 166
California red scale, 257, 912
citrus cutworm, 656
citrus red mite, 257
codling moth, 357, 423
corn earworm, 259, 823
cotton leafworm, 758
cyclamen mite, 431
desert spider mite, 646
European chafer, 766
Fannia canicularis, 611
garden tortrix, 597
greenbug, 722
green peach aphid, 139
holly bud moth, 88
house flies, 20, 908
leaf plum aphid, 228
Mexican bean beetle, 259
onion maggot, 885
peach tree borer, 558
potato leafhopper, 259
red-banded leafroller, 456
resistant spotted alfalfa aphids, 313
rufous scale, 404
serpentine leaf miner, 358
six-spotted leafhopper, 903
soft scales on walnut, 427, 429
thrips on cabbage, 395
two-spotted spider mite, 711, 736
walnut aphid, 430
walnut scale, 528
western subterranean termite, 209
western tussock moth, 764
wireworms, 691
Parathion
hydrolysis of the alkylphosphate bond by rats, cockroaches and alkali, 800
movement of in soils, 382
oral chemotherapeutic activity of in mice, 127
not oxidized in *Chlorella* culture, 59
suspected cases of poisoning in the Mississippi Delta area, 832
susceptibility of to brine shrimp, 780
Parathion, resistance to by
citrus red mite, 233
house fly, 497, 546, 616
Tetranychus atlanticus, 286
Tetranychus cinnabarinus, 285
Tetranychus pacificus, 286
Parathion, toxicity of to
Ceratitis capitata, 235
desert spider mite, 670
five species of tetranychid mites, 521
honey bees, 108

internal parasites of spotted alfalfa aphid, 375
melon fly, 236
pink bollworm adults, 578
Pathogens for control of tobacco insects, 560
Pathology, gross, of American cockroach, 663
Pauridea peregrina, 92
Pectinophora gossypiella, 236, 309, 567, 633, 673
Pediculus humanus humanus, 484, 665, 741, 742
Pemphigus
monophagus, 179
populi-caulis, 179
Pentachlorophenyl, for control of western subterranean termite, 207
Pentatomidae, injury to peaches, 27
Perezia pyraustae, 390
Pergamasus
crassipes, var. *longicornis*, 770
sp. 769
Peridroma margaritosa, 410
Periplaneta americana, 49, 169, 211, 239, 409, 574, 663, 715, 866
Perthane, for control of
black carpet beetle, 249
cabbage leaf-feeding lepidoptera, 443
cherry fruit flies, 594
codling moth, 542
garden tortrix, 597
horn flies, 73
imported cabbageworm, 254
onion maggot, 886
serpentine leaf miner, 358
stable flies, 73
thrips on cabbage, 396
Petroleum distillate in treatments against biting flies on cattle, 620
Phaedonia inclusa, 390
Pheidole megacephala, 147
Phenothiazine, used with systemic insecticides, 794
Philaenus lencophthalmus, 81, 219, 492, 743
Phobocampe sp., 764
Phoridae, 112
Phormia regina, 407
Phorocera
fuscimacula, 764
tachinomoides, 562
Phorodon menthae, 179
Phosdrin, for control of
Aceria tulipae, 70
boll weevil, 670
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 254
codling moth, 379
corn earworm, 823
cotton leafworm, 259
cross-striped cabbageworm, 254
desert spider mite, 670
imported cabbageworm, 254
iris borer, 550
Nysius raphanus, 251
onion maggot, 885
red-banded leafroller, 379
resistant spotted alfalfa aphids, 313
serpentine leaf miner, 358
thrips on cabbage, 396
two-spotted spider mite, 736
western tussock moth, 764
Phosdrin
resistance of citrus red mite to, 233
translocation and persistence of in cacao foliage, 773
Phosdrin, toxicity of to
honey bees, 103
Therioaphis maculata and *Hippodamia*, 377
Phosphatase activity of susceptible and resistant flies and cockroaches, 211
Phosphoramides, chromatographic behavior, partition distribution, and anticholinesterase activity of, 52
Phosphorodithioates, carbamoyl alkyl, as chemotherapeutic agents, 126
Phostex, for control of
leaf curl plum aphid, 229
soft scales on walnut, 429
two-spotted spider mite, 736
Phostex, toxicity of to
honey bees, 103
five species of tetranychid mites, 521
Photography, insect, aids in field, 439
Phyllocharis undulata, 390
Phytocoris, injury in peaches, 28
infuscatus, 28
puella, 28
salicis, 28
Phytomyza ilicis, 245
Phytoseiidae, 366, 770
Pieris rapae, 254, 394, 443, 626
Piezodorus guildinii, 636
Piperonyl butoxide, 76
as synergist for pyrethrins, 742
in treatments against biting flies on cattle, 620
synergistic with rotenone against body lice, 484
toxicity and synergism of mixtures with *cis* isomers of allethrin, 675
Pival in bait against confused flour beetle, 301
Plagiodera versicolora, 390
Plagiognathus spp., 493
Plagiomima cognata, 562
Plants, toxicant producing, introduced into Indonesia, 390
Platencyrtus parkeri, 366
Plathypena scabra, 735
Platynota stultana, 296, 598
Plutella maculipennis, 254, 390, 394, 443
Podisus, 637
Polistes exclamans arizonensis, 465
Pollination
birdsfoot trefoil by honey bees, 494
influence on production of crimson clover seed, 523
low-bush blueberries by honey bees, 544
of passion fruit in Hawaii, 146
Polyplax sp., 130
Popillia japonica, 81, 247, 345
Populations
of citrus bud mite, factors affecting, 657
plum curculio in unsprayed peach orchard, 330
Powder-post beetles, Bostrichid, host records of, 556
Praon palitans, 374
Predators, release of in California against citrus mealybugs, 90
Prionoxystus robiniae, 407
Prodenia
eridania, 637, 838
ornithogalli, 562
sp., 390
Prospaltella spp., 406
Protoparce
quinquemaculata, 78, 560
sexta, 78, 560
Protozoa, disease-producing in *Trogoderma*, 916
Psallus seriatus, 41, 489, 872
Pseudaletia unipuncta, 562
Pseudaphycus
angelicus, 92
perdignus, 92
Pseudococcus
adonidum, 90, 433
brevipes, 905
citri, 90, 480, 773
gahani, 90
maritimus, 90
njalensis, 773
Pseudomonas fluorescens, 59
Pseudopeziza, 220
PS-256, tests against northern fowl mite, 188
Psila rosae, 165
Psilothrips
paradalotus, 451
priesneri, 451
Pterostichus vulgaris, 543
Ptilodactyla serricollis, 81
Pulex irritans, 33
Pyemotidae, 461
Pyrausta nubilalis, 133, 735, 744
Pyrazoxon, resistance of citrus red mite to, 233
Pyrenone, for control of
horn flies, 73
Pagiocerus fiorii, 468
stable flies, 73
Pyrenone, toxicity of in soil to *Ceratitis capitata*, 235
Pyrethrins, for control of
black carpet beetle, 249
body lice, 742
horn flies, 73, 154, 276
Nysius raphanus, 251
stable flies, 73, 154, 270, 276
Pyrethrins
in treatments against biting flies on cattle, 620
synergized, for control of bed bug, 241
toxicity of in house fly sprays, 810
with acetal synergists in house fly sprays, 39
Pyrethrum, toxicity of to *Therioaphis maculata*, and *Hippodamia*, 377
Pyrolan, toxicity of in soil to *Ceratitis capitata*, 235

Radioactive materials
absorption and toxicity in mosquito larvae, 640
carboxy-labeled C^{14}-*N*,*N*-diethyl-*m*-toluamide, 707
cobalt-60 to tag wireworms, 291
dimefox in the cockroach, 50
fate of P^{32}-labeled Bayer 21/199 in the white rat, 204
P^{32} parathion in soils, 380
schradan in the cockroach, 50
(S^{35}) Bayer 19639 in pineapple plants, 905
Raphignathus sp., 366
Rearing, laboratory of the boll weevil, larval diet, 288
Red clover, yield losses caused by clover root borer, 491
Reduviidae, 549
Regression, multiple, to compare several meadow spittlebug insecticides, 743
Repellents
against horn flies and stable flies on beef cattle, 276
insect, synthesis of, 707
insect, ultraviolet determination of diethyltoluamide, 694
insecticide seed treatments against wireworms, 291

Residues
in swine sprayed with malathion, 256
persistence of aldrin on alfalfa, 222
persistence of heptachlor on alfalfa, 222
Residues of
Chipman R-6200 in cacao, 776
commonly used insecticides on alfalfa, 392
DDT and malathion on turnip tops, collards, snap beans, and lettuce, 306
demeton in cacao, 777
demeton on cabbage and celery, 175
dieldrin on alfalfa treated with granules, 685
dieldrin in aldrin-treated carrots, 340
insecticide, in milling fractions in wheat treated with insecticides, 516
insecticide, in soils, 186
insecticides on vegetables, 818
Isolan in cacao, 777
lindane on and in treated wheat, 844
malathion on and in treated wheat, 844
methoxychlor in milk of treated cattle, 618
Neotran on and in lemons and oranges, 914
Thimet in cacao, 777
Thimet in small grains grown in treated soil, 623
toxic non-aldrin in carrots grown on aldrin-treated soil, 337
Residues, bromine
in avocados after fumigation with ethylene chlorobromide and ethylene dibromide, 899
in commodities after fumigation with methyl bromide, 896
Resistance
alfalfa to meadow spittlebug, 219
alfalfa to spotted alfalfa aphid, 457, 760
bed bugs to DDT, 241
boll weevil to toxaphene, 721
citrus red mite to organic phosphates, 232
codling moth to DDT, 693
Colorado potato beetle to DDT, 828
Culex tarsalis to DDT, 56
DDT, attempt to reduce in body lice, 665
difference in damage to varieties of sweet corn by Japanese beetle, 247
effects of chilling pupae on emergence of DDT-resistant house flies, 666
effect of seeding density of spring wheat on wheat stem sawfly, 804
effect of temperature on insecticides against boll weevil, 93
European red mite to phosphorus-based acaricides, 255
genotypic reactions to boll weevil attack in Upland cotton, 508
potato flea beetle to DDT, 823
reproduction in insecticide-treated house flies, 579
spotted alfalfa aphid to organophosphorus insecticides, 312
stable flies to DDT in Norway, 599
sweetclover to sweetclover weevil, 190
Tetranychus to organophosphorous insecticides, 285
to boll weevil in mixed populations of resistant and susceptible cotton plants, 502
winter wheat to the greenbug, 702
Resistance of house fly to
Bayer 21/199, 497
chlordane, 497
dicapthon, 497
DDT, 497, 647
Diazinon, 497, 546, 616
dieldrin, 497
Dipterex, 497, 616
Dow ET-57, 497
lindane, 497
malathion, 18, 452, 497, 546, 616
methoxychlor, 497
organophosphorus compounds, 20, 616
parathion, 497, 546, 616
Reticulitermes hesperus, 207
Rhadinopsylla heiseri, 33
Rhagoletis
completa, 532, 551, 686
cingulata, 592
fausta, 592
Rhopalosiphum
fitchii, 354, 553
maidis, 185, 553, 637
nymphaeae, 227
padi, 179
poae, 179
Rhopobota naevana, control of, 87
Rhyacionia frustrana, 556
Rhyssa persuasoria, 650
Rhyzopertha dominica, 843, 900
Rotenone
for control of Mexican bean beetle, 554
ineffective against variegated cutworm, 410
toxicity of to *Therioaphis maculata* and *Hippodamia*, 377
R-326, for control of stable flies, 270
Ryania, for control of
codling moth, 379, 422
Lithocolletis sp., 423
red-banded leafroller, 379
sugarcane borer, 562

Sabadilla, toxicity of to honey bees, 104
Sabulodes caberata, 296
Safety precautions violated in the use of insecticides, 834
Saissetia oleae, 247
Sanninoidea exitiosa, 557
Sarcophaga, sp., 634, 637
Scambus
boulianae, 650
nucum, 650
Scarabaeid beetles, human ear invasions by, 546
Schistocerca
americana, 345
paranesis, 633
sp., 465
Schizonotus latus, 390
Schoenobius
incertulas, 411, 571
officinale, 390
Schradan
distribution of radioactive, in cockroach, 49
effect of on soft scales on walnut, 429
Schradan, for control of
Aceria tulipae, 70
European red mite, 713
green peach aphid, 139
Nysius raphanus, 251
serpentine leaf miner, 358
walnut aphid, 527
Schradan not oxidized in *Chlorella* cultures, 59
Schradan, resistance of citrus red mite to, 233
Schradan tends to induce increased populations of
calico scale, 527
European red mite, 527
Schradan, toxicity of to *Therioaphis maculata* and *Hippodamis*, 377
Scirtothrips
citri, 451
sp., 451
Scirpophaga
innotata, 390
nivella, 390
Scobicia declivis, 556
Scolothrips sexmaculatus, 451
Scolytus multistriatus, 256, 469, 789
Screens, plastic coated glass fiber insect, 864
Scutellista cyanea, 247
Scymnus
quadrivittatus, 92
sordidus, 90
sp., 637
Seed treatments, cotton with systemic insecticides, 160
Sehirus cinctus, 81
Selenasphidus articulatus, 404
Sesamia inferens, 571
Sevin, effect of on cocooning codling moth larvae, 672
Sevin, for control of
apple aphid, 422
boll weevil, 236, 490, 784
bollworm, 236, 490, 646
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 236, 254
codling moth, 379, 422, 542
corn earworm, 259, 823
cross-striped cabbageworm, 254
cotton aphid, 236
cotton fleahopper, 490
cotton leafworm, 237, 259
imported cabbageworm, 254
Lithocolletis sp., 423
Mexican bean beetle, 259, 554
onion maggot, 885
pink bollworm, 236
potato leafhopper, 259
red-banded leafroller, 379
salt-marsh caterpillar, 237
sugarcane beetle, 633
thrips on cabbage, 396
thrips, 489
western tussock moth, 764
Sevin, tests against northern fowl mite, 189
Sevin (C-7744), toxicity of to
goldfish, 540
honey bees, 103
Shell OS-1808
for control of, *Aceria tulipae*, 70
translocation and persistence of in cacao foliage, 773
Shell OS 1836
for control of onion maggot, 885
translocation and persistence of in cacao foliage, 773
Siglure (ENT 21486) as a lure for Mediterranean fruit fly, 679
Simulium
congareenarum, 845
decorum, 846
slossonae, 845
tuberosum, 845
venustum, 845
verecundum, 845
Siphona irritans, 72, 149, 269, 275, 559, 618, 746, 795
Sitona
cyclindricollis, 190
hispidula, 554, 917
Sitophilus
granarius, 674, 701, 843, 900
oryza, 23, 157, 674, 843, 860, 900, 913

sasakii, 913
Sitotroga cerealella, 23, 674
S-MP powder, tests against northern fowl mite, 188
Sodium arsenite
for control of western subterranean termite, 207
spray injury to dairy cattle by, 539
Sodium pentachlorophenate, for control of western subterranean termite, 207
Soil drenches, insecticidal, effect of on growth of young cabbage and muskmelon plants, 534
Solenopsis saevissima, 111
Solenoptes capillatus, 877
Solvents, organic, effects of on American cockroach, 664
Speed of action, comparative study, of some halogen-containing thiol-alkylating agents on resistant house flies, 880
Spicaria rileyii, 560
Spodoptera mauritia, 571
Sprays, barn, residual, effect of on horn flies, 559
Staphylinidae, 770
Steneotarsonemus pallidus, 431
Stenotarsus hispidus, 81
Stenotus binotatus, injury to peaches, 28
Stomoxys calcitrans, 72, 149, 250, 269, 275, 425, 499, 599, 612, 618, 861
Strobane, for control of
Aceria tulipae, 70
black carpet beetle, 249
European chafer, 766
serpentine leaf miner, 358
Strobane, toxicity of in soil to *Ceratitis capitata*, 235
Sulfoxide
as synergist for pyrethrins, 742
synergistic with rotenone against body lice, 484
Sulfur for control of
mites attacking eye gnat cultures, 462
Nysius raphanus, 251
Sulfur, relative toxicity of to five species of tetranychid mites, 521
Sulphenone, for control of northern fowl mite, 189
Supella supellectilium, 608
Sympherobius spp., 90
Synergism
compounds showing, with rotenone against body lice, 484
of mixtures of piperonyl butoxide with *cis* isomers of allethrin, 675
piperonyl butoxide and IN-930 with pyrethrins against house flies, 742
sulfoxide and IN-930 with pyrethrins against body lice, 742
Synergists
ability of two polyfluoro BHC analogs to synergize lindane, 413
compounds related to DDT evaluated as DDT synergists, 647
Syrbula admirabilis, 345
Syrphus americanus, 81
Sytam, for control of iris borer, 550

Tabanus
giganteus, 618
quinquevittata, 275
sp., 72
sulcifrons, 274, 618
Tabutrex, for control of stable flies, 270
Tachinidae, reared from lepidopterous larvae in Arizona, 561
Taeniothrips orionis, 274
TDE (DDD), for control of
citrus cutworm, 656
codling moth, 379
garden tortrix, 597
red-banded leafroller, 379, 456
serpentine leaf miner, 358
western tussock moth, 764
Technique (see also methods)
aids for field photography of insects, 439
testing insecticide deposits with newly hatched codling moth larvae, 547
Tedion, for control of
citrus red mite, 352
European red mite, 353, 713
lone star tick, 425
mites attacking eye gnat cultures, 462
screw-worm, 425
stable fly, 425
two-spotted spider mite, 352, 736
Teleonemia scrupulosa, 390
Temperature, effect of on
efficiency of insecticides against boll weevil, 93
fumigation, 892
house fly emergence, 666
survival of aphids, 144
survival rate of European red mite eggs, 263
Tenebroides mauritanicus, 866
TEPP, for control of
citrus cutworm, 656
serpentine leaf miner, 358
TEPP, toxicity of to *Therioaphis maculata* and *Hippodamia*, 377
TEPP, used with polyhedrosis virus, for control of cabbage looper, 629
Tetracnemus
peregrinus, 92
pretiosus, 92
Tetranychidae, 41, 633, 767
Tetranychus
atlanticus, 285
bimaculatus, 767
cinnabarinus, 225, 518
desertorum, 518, 634, 645, 872
gloveri, 518
lobosus, 518
ludeni, 519
mcdanieli, 588
pacificus, 225
tetarius, 238, 352, 356, 369, 519, 710, 713
tumidus, 518, 669
Tetrastichus
marcovitchi, 401
sp., 366
whitmani, 401
Thanasimus nigriventris, 604
Thanite, in treatments against biting flies on cattle, 620
Thanite, for control of
horn flies, 73
stable flies, 73
Therioaphis (*Pterocallidium*) *maculata*, 122, 179, 287, 312, 341, 374, 392, 397, 457, 553, 573, 601, 612, 760
Thermal constants of seed-corn maggot, 704
Thermobia domestica, 248
Thimet, effect of seed treatments on cotton, 495, 697
Thimet, for control of
bagworm, 368
balsam gall midge, 400
birch leaf miner, 245
boxwood leaf miner, 245
boll weevil, 100
cabbage leaf-feeding lepidoptera, 443
citrus mealybug, 779
citrus whitefly, 552
codling moth, 422
corn earworm, 259
cotton aphid, 120
European red mite, 714
green peach aphid, 139
holly leafminer, 245
leaf curl plum aphid, 226
Mexican bean beetle, 259
onion maggot, 885
potato leafhopper, 259
Scolytus multistriatus, 789
serpentine leaf miner, 358
spotted alfalfa aphid, 122, 457
sugarcane beetle, 633
thrips on cabbage, 395
thrips on cotton, 160
two-spotted spider mite, 714, 736
Thimet, metabolism of by
plants, insects, and mammals, 838
soil microorganisms, 59
Thimet
tests with as cottonseed treatments, 872
to prevent blue dwarf disease in oats, 623
toxicity and stability to hydrolysis by alkali, 839
toxicity of to honey bees, 103
translocation and persistence of in cacao foliage, 773
Thiobacillus thiooxidans, 59
Thiodan, for control of
boll weevil, 42, 490, 784
bollworm, 42, 490, 646
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 113, 254
citrus whitefly, 552
corn earworm, 259, 823
cotton fleahopper, 490
cross-striped cabbageworm, 254
greenbug, 722
imported cabbageworm, 254
lone star tick, 425
Mexican bean beetle, 259, 554
onion maggot, 885
potato leafhopper, 259
screw-worm, 425
serpentine leaf miner, 358
soft scales on walnut, 430
stable fly, 425
sugarcane beetle, 633
thrips, 395, 489
two-spotted spider mite, 736
walnut scale, 530
Thiol alkylating agents, halogen-containing, speed of action on resistant house flies, 880
Thrassis bacchi gladiolis, 32
Thrips
control of on cotton with systemics, 698
effect of on height and weight of seedling cotton, 115
hawaiiensis, 147
of Arizona, 450
tabaci, 394, 451
Thyanta accumulata, 636
Thyridopteryx ephemeraeformis, 367
Thysanus
merceti, 114
thoreauini, 114
Timberlakia europaea, 365
Tineola bisselliella, 248, 919
Tomocera (*Moranila*), 247
Torulopsis utilis, 59
Toxaphene
effect of wind and rain on toxicity of to boll weevil, 670
effect on insect predators on alfalfa, 615

Toxaphene, for control of
Aceria tulipae, 70
alfalfa pests, 730
alfalfa snout beetle, 683
boll weevil, 490, 784
bollworm, 490, 646
cabbage leaf-feeding lepidoptera, 443
cabbage looper, 113, 254
citrus cutworm, 656
clover leaf weevil, 195
Colorado potato beetle, 829
corn earworm, 823
cotton fleahopper, 490
cotton leafworm, 259, 784
cross-striped cabbageworm, 254
cyclamen mite, 432
European chafer, 766
greenbug, 722
horn fly, 795
imported cabbageworm, 254
meadow spittlebug bug, 493
Nysius raphanus, 251
seed-corn maggot, 919
serpentine leaf miner, 358
thrips, 396, 489, 698
two-spotted spider mite, 736
weevils on crimson clover, 459
western subterranean termite, 209
western tussock moth, 764
Toxaphene
residues of on vegetables, 818
residues in treated soil, 187
spray compared with cottonseed treatments with systemics, 873
susceptibility of the boll weevil to, 719
tests against boll weevil, 95
Toxaphene, toxicity of to
buckthorn aphid, 414
foxglove aphid, 414
green peach aphid, 414
pink bollworm adults, 568
potato aphid, 414
Therioaphis maculata and *Hippodamia*, 377
Toxoptera
graminum, 553, 701, 722
muklenbergiae, 179
Toxorhynchites rutilus, 744
Transmission of sugar beet yellow-net virus, latent period phenomena in, 812
Traps
for eradicating Mediterranean fruit fly in Florida, 679
for walnut husk fly, 689
light, insects attracted to, 550
light, mechanical and suction-type for hornworm moths, 78
Triatoma sanguisuga, 549
Tribolium confusum, 239, 299, 345, 385, 409, 674, 860, 900
Trichoplusia ni, 113, 236, 254, 296, 394, 443, 562, 626
Triozys utilis, 374
Trithion, for control of
California red scale, 912
codling moth, 422, 542
corn earworm, 259
desert spider mite, 646
European red mite, 255, 422, 714
leaf curl plum aphid, 228
lone star tick, 425
Mexican bean beetle, 259
northern fowl mite, 189
onion maggot, 883
paddy borer, 411
potato leafhopper, 259
resistant spotted alfalfa aphids, 313
screw-worm, 425
soft scales on walnut, 430
stable fly, 425
two-spotted spider mite, 710, 714, 736
walnut scale, 530
Trithion, resistance of
citrus red mite to, 232
Tetranychus atlanticus to, 286
Tetranychus pacificus to, 286
Trithion, toxicity of to
honey bees, 103
Therioaphis maculata and *Hippodamia*, 377
Trogoderma
glabrum, 916
granarium, 465, 900, 916
inclusum, 916
parabile, 916
simplex, 916
sternale, 763, 916
teukton, 916
Trogus, 652
Trombidiidae, 771
Trombidiformes, 768
Tropidophryne melvillei, 92
Tyndarichus sp., 366

Udea rubigalis, 296
Urginea maritima, 390
Uropodina, 771

Vanessa cardui, 562
Vasates
cornutus, 337
fockeui, 335
V-C 13, for control of onion maggot, 886
Vectors, yellow-fever, in Dallas, Tex., 743
Veigaia
cerva, 770
nemorensis, 770
Vermiculite
as a carrier for insecticides against wireworms, 463
use of in medium for stable fly larvae, 250
Virus disease
Aceria tulipae as vector of, 68
granulosis, for control of red-banded leaf roller, 454
polyhedrosis, for control of cabbage looper, 626
sugar beet yellow-net, transmission of by green peach aphid, 812
Volucella obesa, 147
Voria ruralis, 562

Warfarin, ineffective against confused flour beetle, 302
Water drinking by larvae of European corn borer, 744
Weather
effect of on toxicities of insecticides, 669
role of in natural control of insect populations, 474
Wheat, development of weevils and fungi in columns of, 701
Winthemia sp., 637

Xanthoencyrtus phragmitis, 365
Xenopsylla cheopis, 239
Xylocopa varipuncta, 146
Xylomyges curialis, 653

Yeast hydrolysate, influence of on melon fly, 140
Yields
corn, reduction of by southwestern corn borer, 747
red clover, effect of distance from honey bee colonies on, 64

Zarhopalus sheldoni, 92
Zeadiatreae grandiosella, 747
Zenillia hyphantriae, 562
Zercoseius sp., 366

SD
1
J8

THE UNIVERSITY OF MICHIGAN
JAN 28 1959
FORESTRY LIBRARY

PR

Journal of ECONOMIC ENTOMOLOGY

Published by the
ENTOMOLOGICAL SOCIETY OF AMERICA

VOLUME 51 December · 1958 NUMBER 6

JOURNAL OF ECONOMIC ENTOMOLOGY

Published by
ENTOMOLOGICAL SOCIETY OF AMERICA

F. W. Poos, *Editor*, 1530 P St., N.W.,
Washington 5, D.C.

EDITORIAL BOARD

F. S. Arant, *Chairman*, Auburn, Ala. (Section F, 1958)

E. L. Chambers, Madison, Wis. (Section E, 1959)

R. L. Metcalf, Riverside, Calif. (Section B, 1960)

D. E. Howell, Stillwater, Okla. (Section D, 1961)

G. J. Haeussler, Beltsville, Md. (Section C, 1962)

A bimonthly journal published on the 15th of February, April, June, August, October, and December, devoted to the interests of economic entomology. The authors are entirely responsible for statements in the *Journal*, whether of fact or opinion.

TERMS OF SUBSCRIPTION. Non-members in North America and U.S. possessions—$12.00; elsewhere —$12.00 plus 60¢ postage. Single copies $2.25 plus postage of 10¢ per copy. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk. LOST NUMBERS. Missing numbers cannot be supplied free of charge unless notice is received within 60 days after the appearance of issues concerned.

REPRINTS. No gratis copies are supplied. A schedule of prices which will apply to orders received before the *Journal* is printed, and an order form are sent with proof. For price schedule see Vol. 1, No. 4, December, 1955 of the Bulletin of the Entomological Society of America, or page 577 of Vol. 49(4) of the *Journal*.

BUSINESS COMMUNICATIONS of the Society and SUBSCRIPTIONS AND MATTERS PERTAINING TO ADVERTISEMENTS should be sent to the Executive Secretary, Entomological Society of America, 1530 P Street, N.W., Washington 5, D.C. Change of address must reach the office of the Executive Secretary 5 days prior to the month of issue to be effective for that month. Members should make use of postal regulations providing for forwarding of second class mail by guaranteeing postage upon delivery.

MANUSCRIPTS and other copy for the *Journal* as well as all correspondence concerning *Journal* content, should be addressed to the editor. Prompt publication of acceptable matter may be secured by payment of partial cost of printing and engraving ($16.00 per page plus cost of engravings with additional charge for excessive tabular matter). Cost of engravings in excess of $15.00, for each eight printed pages or major fraction thereof, will be charged to the author. Before preparing manuscripts for publication in the *Journal*, authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions given in Vol. 49(4): 575-77, August, 1956.

Entered as second class matter at the Post Office at Menasha, Wisconsin. Acceptance for mailing at special rate of postage under the provisions of Sec. 34.40, Par. (D), provided for in the Act of February 28, 1925, embodied in paragraph 4, Section 538, P. L. and R., authorized May 1, 1936. Second-class postage paid at Menasha, Wis.

Printed by the George Banta Co., Inc.
Menasha, Wisconsin, U.S.A.

Issued January 19, 1959

1957 NATIONAL ENTOMOLOGY WINNERS: Joe W. Simmons, California; Larry Ulmer, Jr., Delaware; David C. Johnson, Virginia; Paul Mayfield, Vice-President, Hercules Powder Company; Howard E. Breland, Mississippi; Jessie Malone, Jr., Montana; Jack Jewell, Kansas.

COLLEGE SCHOLARSHIPS OFFERED IN 1958 ENTOMOLOGY PROGRAM

To stimulate increased interest in entomology, Hercules Powder Company announces its participation in the 4-H Entomology Awards Program for the seventh consecutive year.

More than 200,000 boys and girls have become better acquainted with the entomological profession as a result of their taking part in this program. The enthusiastic support of all those associated with the advancement of entomological knowledge has been an important contribution to the success of this important youth activity.

The following awards are being offered during 1958:

COUNTY WINNERS: Gold-filled entomology medals.

STATE WINNERS: Trips to the '58 National 4-H Congress.

NATIONAL WINNERS: Six college scholarships of $400 each.

Agricultural Chemicals Division, Naval Stores Department

HERCULES POWDER COMPANY

INCORPORATED

900 Market Street, Wilmington 99, Delaware

Producers of toxaphene and other basic chemicals for agriculture

NX58-1

for maximum effectiveness in insecticide spraying

Year after year, Spraying Systems spray nozzles and spray guns demonstrate their superiority in the spraying of every type of liquid insecticide. Reason for this is advanced hydraulic design plus precision in manufacture. Uniform spray distribution, exact control of volume, and accurate spray pattern are a few of the dependable advantages of Spraying Systems equipment. The favorite spray nozzles and spray guns in experimental work, Spraying Systems TeeJets, Trigger TeeJets and GunJets offer maximum reliability in commercial and government controlled insecticide spraying. Write for information on these Spraying Systems products:

TeeJet Spray Nozzles

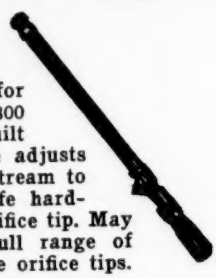
with interchangeable orifice tips

Supplied with male or female connection, in choice of over 400 interchangeable orifice tips. Give full range selection to meet any requirement. Flat spray, hollow cone, full cone and straight stream types. For all liquids including those with suspended solids. For use with boom or gun type sprayers.

Trigger TeeJet Shut-Off Valves

For use at any pressure up to 250 pounds. Light in weight and easily handled. Fits comfortably in hand. Positive shut-off valve with lock. For use with all TeeJet interchangeable orifice tips. Supplied with or without large capacity strainer in handle.

GunJet Spray Guns



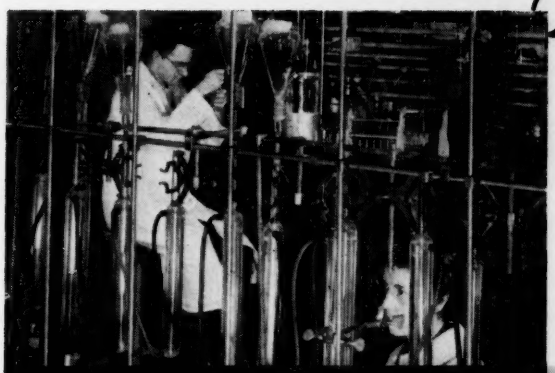
Precision built guns for pressures from 30 to 800 pounds. Ruggedly built for hard use. Handle adjusts spray from straight stream to hollow cone. Long life hardened stainless steel orifice tip. May also be used with full range of TeeJet interchangeable orifice tips.

Spraying Systems products are sold by original equipment manufacturers everywhere as a part of the equipment they build . . . or are available directly from Spraying Systems Co. for application in experimental work. Products also include strainers and fittings required in spraying.

FOR INFORMATION . . . write for general catalog No. 30 . . . Bulletins 66 and 71, BoomJet Spray Nozzles . . . Bulletins 65, 69 and 80, GunJet Spray Guns . . . and Bulletin No. 68, Trigger TeeJet Shut-Off Valves.

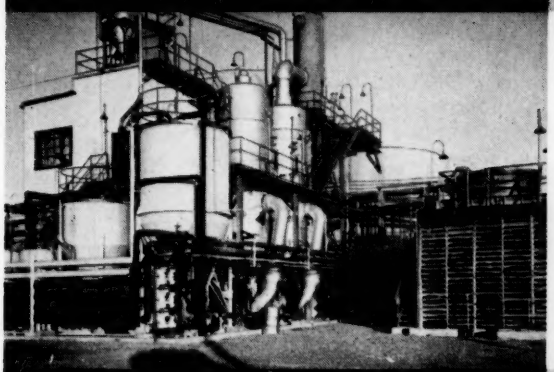
SPRAYING SYSTEMS CO., 3218 Randolph Street, Bellwood, Illinois

What does it really take to kill insects?



RESEARCH . . . laboratory work that takes many years and millions of dollars

TESTING . . . years of field work, to determine effectiveness, dosages, side effects



PRODUCTION . . . to make commercial quantities available at an economically sound cost

EDUCATION . . . teaching methods of use, gaining confidence and trust

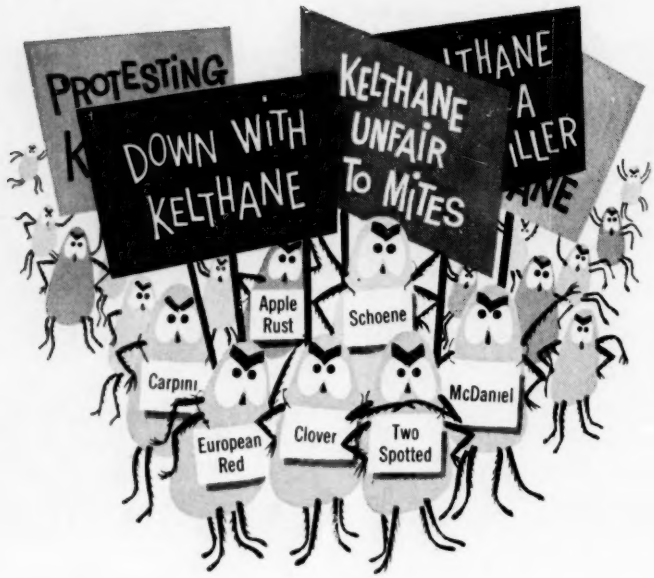
A great deal of work and knowledge goes into the development of insecticides. Once developed, however, they're a priceless defense against the loss of food and fiber, the disease, and the annoyance caused by man's insect enemies. Velsicol Chemical Corporation manufactures five basic insecticides that are thoroughly proven, widely recommended, and extensively used: Heptachlor, Chlordane. Endrin, Parathion and Methyl Parathion. Each of them is rated most efficient for specific types of insect control, and all are available everywhere, in ready-to-use, reasonably priced formulations. If you deal with any phase of insect control, we think you will find technical information about these insecticides highly useful.

NEW! VELSICOL GIBBERELLINS!
Amazing plant growth stimulant. Velsicol's newest contribution to increased agricultural productivity. Extensive research program in progress.

WRITE FOR A BROCHURE OF TECHNICAL INFORMATION, PREPARED BY VELSICOL'S ENTOMOLOGISTS!
HEPTACHLOR • CHLORDANE • ENDRIN • PARATHION • METHYL PARATHION

VELSICOL

VELSICOL CHEMICAL CORPORATION
330 East Grand Ave., Chicago 11, Illinois
International Representative: Velsicol International Corporation, C.A.
P.O. Box 1687 • Nassau, Bahamas, B.W.I.



Count on KELTHANE to kill mites and prove to yourself the truth of this statement: "With KELTHANE, you get low-cost and the *best* mite control." The money-saving reasons for this high-activity and low-cost performance are all-important:

- KELTHANE delivers quick and certain kill to most of the troublesome mite species.
- KELTHANE has l-o-n-g—l-a-s-t-i-n-g residual action; therefore, fewer applications are needed.
- KELTHANE is safe on foliage, safe to fruit finish, and safe to handle, when used as recommended.
- KELTHANE is cleared for use on over forty different fruit, vegetable, and field crops.

Begin your KELTHANE program as soon as mites start to build up. You will like the way KELTHANE kills mites in hot or cool, humid, wet, or dry weather. You can use KELTHANE all season—to within a few days of harvest if necessary.

Chemicals for Agriculture

ROHM & HAAS COMPANY

WASHINGTON SQUARE, PHILADELPHIA 5, PA.

Representatives in principal foreign countries

KELTHANE *is a trademark, Reg. U.S. Pat. Off. and in principal foreign countries.*

CHECKMATE FOR MITES

KELTHANE

Easy-to-apply Shell Chemical insecticides give dependable control.

Gone are the "loss and buggy" days!

The days of farmers' complete crop losses to "bugs" are rapidly disappearing and will soon be only a memory like the horse and buggy. Today's insecticides give a decided advantage to farmers. With dependable insect control, they now market a much larger share of harvests.

To discover newer and better means of protecting crops, Shell Chemical Corporation researchers work with State and Federal experimental stations, independent researchers, extension workers and growers.

As a result of intensive experimentation, Shell developed aldrin, dieldrin, endrin and Phosdrin® insecticides; D-D® and Nemagon® soil fumigants; and Allyl Alcohol weed seed killer.

Other pesticides being developed in experimental installations throughout the country promise even greater success for agriculture—and a bleak future for insect pests!

Technical information on Shell Chemical Corporation pesticides is available. Write to:

SHELL CHEMICAL CORPORATION

AGRICULTURAL CHEMICAL SALES DIVISION
460 PARK AVENUE, NEW YORK 22, NEW YORK

SHELL

Two New Insecticides From ORTHO
ORTHO PHOSPHAMIDON—ORTHO DIBROM

ORTHO PHOSPHAMIDON

ORTHO PHOSPHAMIDON 4 Spray is a water soluble liquid systemic insecticide with moderate residual for the control of both sucking and chewing insects. ORTHO Phosphamidon 4 Spray is suggested for trials on various sucking, chewing, boring or mining insects on cotton, fruits, vegetables, forage crops and ornamentals. ORTHO Phosphamidon 4 Spray has proven unusually effective against aphids, boll weevils and codling moths. Limited quantities of ORTHO Phosphamidon 4 Spray are available for experiment station testing.

ORTHO DIBROM

ORTHO DIBROM is an insecticide with control properties for a wide range of insects. It is especially effective against Lepidoptera and Diptera. ORTHO Dibrom has a short residual, low mammalian toxicity, indicating useful applications close to harvest. ORTHO Dibrom 8 Emulsive and Dusts are now available for extensive field trials.

Answers to technical questions concerning ORTHO Phosphamidon and ORTHO Dibrom may be obtained by addressing inquiries originating in the eleven Western States to Dr. Thompson . . . in the Midwest and East to Dr. Wessel:

DR. RICHARD WESSEL
Field Research Supervisor, East
California Spray-Chemical Corp.
Box 118, Moorestown, N.J.

Dr. R. K. THOMPSON
Field Research Supervisor, West
California Spray-Chemical Corp.
Lucas and Ortho Way, Richmond 4, Calif.

On all chemicals, read directions and cautions before use

CALIFORNIA SPRAY-CHEMICAL CORPORATION
A Subsidiary of California Chemical Company

EXECUTIVE OFFICES:
RICHMOND, CALIFORNIA • WASHINGTON, D.C.

District Offices: Portland 22, Oregon, Box 5946 • Sacramento 10, Calif., Box 2227 • San Jose 8, Calif., Box 978 • Fresno 7, Calif., Box 48 • Whittier, Calif., Box 471 • Phoenix, Ariz., Box 11068 • Salt Lake City 10, Utah, Box 2070 • Maryland Heights, Mo., Box 129 • Shreveport, La., Box 1164 • Memphis 11, Tenn., Box 6405 • Maumee, Ohio, Box 239 • Haddonfield, N.J., 133 Kingshighway East • Springfield 9, Mass., Box 402 Highland Station • Medina, N.Y., Box 230 • Columbia, S.C., Box 576 • Orlando, Fla., Box 7067.

BRANCH OFFICES THROUGHOUT THE UNITED STATES

TM'S ORTHO, REG. U.S. PAT. OFF.

ORTHO®

"Helping The World Grow Better"

CLASSIFIED ADVERTISEMENTS

Rates for classified advertisements are 10¢ per word, with a $2.00 minimum charge. An exception is made for individuals seeking employment where the rate is 5¢ per word, with a $1.00 minimum. Closing date is the 10th of the month preceding month of issue. Address all replies to Classified Advertisements having a Box Number to the Entomological Society of America, 1530 P Street, N.W., Washington 5, D.C.

FOR SALE. Rare terra cotta hand painted exotic butterflies. Faithfully reproduced in full size and the shape and colors of living butterflies. For information and prices, write to Isofrance, 27bis, passage MERMET, LYON (1°) FRANCE.

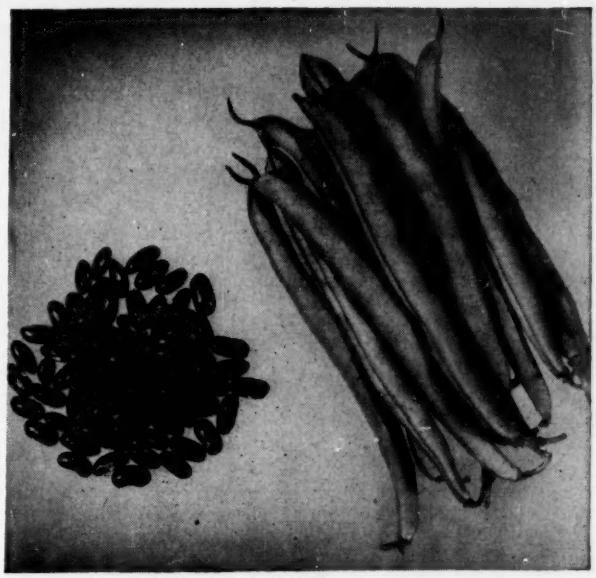
TECHNICAL PERSONNEL wanted by large service-minded pest control company. Duties to assist branch offices in pest and termite control work. Includes training and direct assistance. No sales. Must have ability to deal with people and willing to do some travel. Experience helpful but not essential. Benefits include life insurance, hospitalization, retirement, paid vacation, and sick leave. Ample opportunity for advancement. Send resume including salary history. All replies confidential. Interviews in Atlanta by invitation at our expense. Write in detail to ORKIN EXTERMINATING CO., INC., 713 WEST PEACHTREE ST., ATLANTA, GA., ATTENTION: TECHNICAL DEPARTMENT.

ENTOMOLOGIST: Position open in San Francisco Bay Area for Entomologist with B.S. or M.S. for research work in development of pesticides. Send personal data including photograph, education and training. Box A, Entomological Society of America, 1530 P Street, N.W., Washington 5, D.C.

ECONOMIC ENTOMOLOGIST Ph.D. A grade; over 10 years experience in Applied Research and Pest-control, held posts as leaders of projects; seeks research or teaching job in colleges, experiment stations or industry. Box B, Entomological Society of America, 1530 P Street, N.W., Washington 5, D.C.

INDEX TO THE LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY. Fifteen volumes, all bound in black buckram. Indexes the literature for 50 years—1905 through 1955. Price $60.00 postpaid in North America and U. S. Possessions. Postage added elsewhere. The Entomological Society of America, 1530 P Street, N.W., Washington 5, D.C.

Effective
MITE KILL
at lower cost per acre



with ARAMITE

Aramite, today's safest strongest mite-killer, promises growers bigger, more profitable yields of better fruits, vegetables, row crops, cotton and ornamentals. This is possible because Aramite can effectively control a wide variety of mites at low cost per acre without harming humans, animals or crops. Further advantages of Aramite are ease of use, long residual effect, high compatibility and harmlessness to natural predators.

US RUBBER **United States Rubber**

Naugatuck Chemical Division
Naugatuck, Connecticut

producers of seed protectants, fungicides, miticides, insecticides, growth retardants, herbicides: Spergon, Phygon, Aramite, Synklor, MH, Alanap, Duraset.

Pyrenone* TODAY'S VERSATILE INSECTICIDE BASE

for insect control without hazard to humans and animals

Safer, more effective for:

LIVESTOCK
DAIRY
FOOD PROCESSING
FOOD HANDLING AND STORAGE
AEROSOLS
HOUSEHOLD USES
STORED GRAINS

Pyrenone (technical *piperonyl butoxide* and *pyrethrins*), rotenone and pyrethrum are exempt from the requirements of a tolerance under the Miller Bill. They may be used right up to the time of harvest.

Pyrenone Wheat Protectant, Pyrenone Grain Protectant and Pyrenone liquid protectants can be applied to wheat, corn (including popcorn), buckwheat, barley, rye and rough rice, in accordance with package directions, for protection during a normal period of storage with a *single* application.

UNDER THE MILLER BILL *piperonyl butoxide* has an approved tolerance of 20 p. p. m. and *pyrethrins* a tolerance of 3 p. p. m. These tolerances are in *excess* of the quantities specified for use in the package directions.

*Reg. U.S. Pat. Off., F.M.C.

FAIRFIELD CHEMICALS

TRADE MARK

FAIRFIELD CHEMICAL DIVISION
Food Machinery and Chemical Corporation
441 Lexington Ave., New York 17, N.Y.
Branches in Principal Cities

fmc ®

In Canada: Natural Products Corporation: Toronto and Montreal

THOMAS SAY FOUNDATION

VOLUME I Sarcophaga and Allies of North America. By J. M. Aldrich. $4.50

VOLUME II The Plecoptera or Stoneflies of America North of Mexico. By J. G. Needham and P. W. Claassen. $5.00

VOLUME III Plecoptera Nymphs of North America. By P. W. Claassen. $4.00

VOLUME IV The Blow Flies of North America. By D. G. Hall. $6.50

VOLUME V Aphids of the Rocky Mountain Region. By Miriam A. Palmer. $10.25

All volumes are cloth bound and all are well illustrated. Volume IV has five colored plates, and Volume V has eight.

Outside North America and U. S. Possessions, add 50¢ per volume to cover postage and handling. Registration or insurance at purchaser's expense.

Entomological Society of America
1530 P Street, N. W.
Washington 5, D. C.

PREPUBLICATION ANNOUNCEMENT
ANNUAL REVIEW OF ENTOMOLOGY

VOLUME 4

EDITOR: E. A. STEINHAUS ASSOCIATE EDITOR: R. F. SMITH

Editorial Committee: ROBERT GLEN, R. L. METCALF, H. H. ROSS, L. E. ROZEBOOM, E. A. STEINHAUS, C. M. WILLIAMS

Volume 4 of this series will appear about February 1, 1959. The three previous volumes, which are still available, established the high standards of the ANNUAL REVIEW OF ENTOMOLOGY.

Members of the Entomological Society of America. Your order should be placed with our Washington office. *Every member should have a copy of the Review.*

TABLE OF CONTENTS

Industrial Melanism ..... H. B. D. Kettlewell
Taxonomic Problems with Closely Related Species ..... W. J. Brown
Insect Blood Cells ..... V. B. Wigglesworth
Population Dynamics ..... T. Burnett
Biology of Fruit Flies ..... L. D. Christenson and R. H. Foote
Bioclimatic Studies of Insects ..... P. S. Messenger
Biology of Aphids ..... J. S. Kennedy and H. L. G. Stroyan
Insect Behavior and the Concepts of Modern Ethology ..... G. P. Baerends
Culture of Insect Tissues ..... M. F. Day and T. D. C. Grace
Insect Pigments ..... R. I. T. Cromartie
Insect Respiration ..... H. A. Schneiderman
Ectohormones in Insects ..... A. Butenandt and P. Karlson
Mode of Action of Insecticides ..... P. Winteringham and S. E. Lewis
Bio-Assay of Insecticide Residues ..... S. Nagasawa
Fruit Insects and their Control ..... M. M. Barnes
Seed Treatment for Insect Control ..... W. H. Lange
Biology of Parasitic Hymenoptera ..... R. L. Doutt
Biological Control of Weeds with Insects ..... C. B. Huffaker
Fleas and Disease ..... W. L. Jellison
Insects and the Epidemiology of Malaria ..... P. F. Russell
Microbial Control ..... Y. Tanada
Biology of Cerambycidae ..... E. Gorton Linsley

Libraries, companies, other organizations and non-members should place their orders with: Annual Reviews Inc., Grant Avenue, Palo Alto, California. Price to organizations and non-members: $7.00 per copy postpaid in the U.S.A., $7.50 per copy elsewhere.

ENTOMOLOGICAL SOCIETY OF AMERICA
1530 P Street, N.W.
Washington 5, D.C.

ENTOMOLOGICAL SOCIETY OF AMERICA

OFFICERS FOR 1958

President—R. L. METCALF, Riverside, Calif.

President-elect—P. W. OMAN, Beltsville, Md.

Executive Secretary—R. H. NELSON, 1530 P St., N.W., Washington 5, D.C.

GOVERNING BOARD*

R. L. METCALF, Riverside, Calif. (1959)

H. M. ARMITAGE, Sacramento, Calif. (1958)

J. E. BUSSART, Chicago, Ill. (North Central, 1960)

C. P. CLAUSEN, Riverside, Calif. (C, 1958)

F. W. FLETCHER, Midland, Mich. (F, 1958)

M. P. JONES, Washington, D.C. (E, 1959)

K. L. KNIGHT, Washington, D.C. (D, 1959)

E. G. LINSLEY, Berkeley, Calif. (A, 1960)

E. H. LITTOOY, Sausalito, Calif. (Pacific, 1959)

C. M. MEADOWS, Waco, Tex. (Southwestern, 1961)

R. H. NELSON, Washington, D.C. (non-voting)

P. W. OMAN, Beltsville, Md. (1960)

B. B. PEPPER, New Brunswick, N.J. (Eastern, 1958)

MORRIS ROCKSTEIN, New York, N.Y. (B, 1960)

O. I. SNAPP, Fort Valley, Ga. (Cotton States, 1959)

* Section or Branch represented and year term expires shown in parentheses.

Branch Chairmen

LESLIE M. SMITH, Davis, Calif.—(Pacific Branch)

C. G. LINCOLN, Fayetteville, Ark.—(Cotton States Branch)

NEELY TURNER, New Haven, Conn.—(Eastern Branch)

R. E. HILL, Lincoln, Nebr.—(North Central Branch)

PAUL GREGG, College Station, Tex.—(Southwestern Branch)

Branch Chairmen-Elect

H. C. MANIS, Moscow, Idaho—(Pacific Branch)

F. E. GUYTON, Auburn, Ala.—(Cotton States Branch)

R. I. SAILER, Beltsville, Md.—(Eastern Branch)

J. W. APPLE, Madison, Wis.—(North Central Branch)

M. A. PRICE, College Station, Tex.—(Southwestern Branch)

Branch Secretaries

H. H. KEIFER, Sacramento, Calif.—(Pacific Branch)

M. E. MERKL, Leland, Miss.—(Cotton States Branch)

BYRLEY F. DRIGGERS, New Brunswick, N.J.—(Eastern Branch)

C. W. WINCO, Columbia, Mo.—(North Central Branch)

HERMAN MEADOWS, Houston, Tex.—(Southwestern Branch)